

3rd Year Report

Susana Roman Garcia

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Table of contents

Preface	4
This is a Quarto Book:	4
Different modes to view this report	5
1 What is my PhD research question?	6
1.1 An overview:	6
1.2 Why use Computational Modelling to study biological systems?	8
2 Ethics and Reproducibility emphasis in this PhD and why it matters	9
2.1 Science for the profit of whom?	9
2.1.1 Historical oppressive biases:	10
2.2 Slowing down...	10
2.3 Importance of reproducibility	11
2.3.1 Defining reproducibility vs replicability	11
2.4 Ethics and Reproducibility go together	12
3 Data Hazards	15
3.1 Example label: high environmental cost	15
3.1.1 Description	15
3.1.2 Examples	16
3.1.3 Safety Precautions	16
3.2 How to use the Data Hazards Project	16
3.3 Application example into Research life-cycle	17
3.3.1 Design:	17
3.3.2 Data Collection and Analysis:	18
3.3.3 Reporting:	18
3.4 Application into my PhD project: Presenting my PhD as a case study at AI UK conference	18
3.4.1 Results from collaborative reflections:	19
3.5 Data Hazards Workshops	23

4 Computer models of CaMKII/NMDAR interactions	27
4.1 Software used: BioNetGen and MCell	27
4.1.1 BioNetGen and how rule-based modelling can help with combinatorial complexity.	27
4.1.2 MCell (Monte Carlo Cell) and how it simulates reactions in 3D	29
4.2 Model description	30
4.2.1 Model of CaMKII as a dodecamer	31
4.3 Model development and validation	31
4.3.1 A reproducible model	32
5 Activities	33
6 Thesis layout	34
References	35

Preface

This year has been mostly focused on building up knowledge on how to put into action the idea of making a more ethical and reproducible PhD. In order to understand the progress I have made this year, I describe in the following chapters the importance of thinking about ethics and reproducibility, what are and how to use Data Hazards, as well as a breakdown of creating reproducible computer models.

At this point in time I am in a place where I have started writing my thesis in ways which allow me to keep good version control, which helps manage the source code and documents by keeping track of all the version modifications. In fact, I am using this year's annual report as an exercise to write an example book using Quarto, which allows for a dynamic implementation of markdown files and python code all in one project, which can be version controlled via Git.

This is a Quarto Book:

This document is written using a Quarto book. Quarto allows for a dynamic implementation of different types of files that can be version controlled, which is extremely helpful to create a pipeline of work all in one place and that is traceable too.

Quarto® is an open-source scientific and technical publishing system built on [Pandoc](#). It allows you to weave together narrative text and code to produce elegantly formatted output as documents, web pages, blog posts, books and more.

Quarto is at its core multi-language and multi-engine (supporting Knitr, Jupyter, and Observable today and potentially other engines in the future); where you can have all your code and narrative text in one. For a full breakdown and FAQs of how Quarto works, you can have a look [here](#).

The good thing for me personally, is that, thanks to Quarto, I can write my thesis chapters using [markdown](#) -which is a lot more intuitive than LaTeX, in my opinion. Moreover, it allows for much better version control compared to Microsoft Word, and allows for helpful traceability of materials included in the document I am writing; which in itself has many benefits as it immensely helps to be able to go back to a previous version where everything was working before I broke the code (yet again).

Part of my work includes work which runs with different python scripts, and using Quarto means that I can embed python code if needed to explain how certain functionalities of the models work. It also allows for code using R to run within a Quarto document, as well as jupyter notebooks, and includes HTML implementations, which can all help with functionalities such as creating tables, adding images, and rendering the document in different formats (website html, or pdf, for example).

Different modes to view this report

- **In an internet browser:** through this [url](#).
- **In .pdf format:** If you are viewing this document in your browser through a url, you can click on the top left icons of the document to download it.
- **Source code in GitHub:** You can also click on the top left icons of the document to access the [source code](#).

1 What is my PhD research question?

The biological question that started this PhD was how do Calcium/calmodulin-dependent protein kinase II (CaMKII) and N-methyl-D-aspartate receptor (NMDARs) in the postsynaptic neuron interact and enable the processes of learning and memory?

But as time went by, I realised more and more how important looking at the ethics of the research that we do is. How biased science actually is, and how we continue to carry these if we don't look at them in the face. Additionally, how much research time and money is wasted by doing experiments which cannot be reproduced or replicated later on. Hence my emphasis on these topics.

I have made it a purpose of this PhD project to highlight and talk about some of the things I care most in research: making it transparent, inclusive, and accessible.

1.1 An overview:

When studying learning and memory at the molecular level, in health and disease, it has been shown that NMDAR and CaMKII together with their interactions with other proteins within neuronal spines, can influence their shape and size ([Fink and Meyer 2002](#)). Long-term modifications of synaptic strength, such as LTD (Long Term Depression) and LTP (Long Term Potentiation) involve diverse chemical pathways and have been the primary mechanisms used to study the molecular basis of learning and memory ([Blundon and Zakharenko 2008](#)). So what exactly is happening at the cellular and molecular level during memory formation?

These are the biological prompts that I look at when creating 3D models of the molecules in question. I use mainly `mcell` and python to do so. In order to give you a better overview of the aims, types of data used, methods and applications of this research, please see below Table 1.1. In addition to the biological aspects of this PhD, as mentioned above, I have made a big effort into making my PhD accessible, reproducible and more ethical. It has transformed into a case study example of how to establish procedures for more ethical and reproducible research, which means future researchers can efficiently re-use and build up on what I have created.

Table 1.1: PhD overview

Wide-view angle of this PhD project
Aims of this PhD:
- Explain how specific molecules work together during memory. - Develop new ways of 3D modelling to look at time and space dynamics of molecular interactions. - Bring awareness of the importance of implementing ethics and reproducibility into a PhD.
Type of data used:
- Kinetic rates of molecule interactions, molecular concentrations collected from literature and databases. - Numbers obtained from either wet-pab experiments or mathematically calculated.
Methods:
- Models written with standardised open source languages: python, bionetgen Language. - Numbers obtained from either wet-pab experiments or mathematically calculated. - Run locally or in cluster if simulations are more computationally expensive.
Applications and significance:
- Other researchers can build from these models to create further predictions for potential pharmacological applications. - Dysregulation of the molecules I look at have been suggested to have a potential impact in Alzheimer's disease, as well associated with multiple forms of spineopathies (Ghosh and Giese 2015), (Robison 2014).

So that you can get a better idea of what the modelling might look like, I drew Figure 1.1, which shows a somewhat simplified version of what the graphical user interface of Cell-Blender can look like. It includes molecules and reactions going in, as well as placement in a 3D cell.

TLDR: I create 3D models which simulate interactions between CaMKII and NMDAR in the postsynaptic neuron, to understand how memory works in animal brains.

1.2 Why use Computational Modelling to study biological systems?

Some of the main reasons for using modelling are:

1. Biological systems are complex and multiscale, models can help us to integrate experimental data, facilitating theoretical hypotheses, and addressing what if questions.
2. Models aim to make clear the current state of knowledge regarding a particular system, by attempting to be precise about the elements involved and the interactions between them. Doing this can be an effective way to highlight gaps in understanding.
3. Related to point one, models then serve to combine knowledge from different published research, and make biological predictions which can then serve as hypothesis to be tested empirically by experimentalists.
4. Computer-simulated experiments can help guide the wet-lab process by narrowing the experimental search space, enabling more cost, time-effective and waste-free research, as well as more ethical research too as we reduce animal suffering through reduction of animal research.

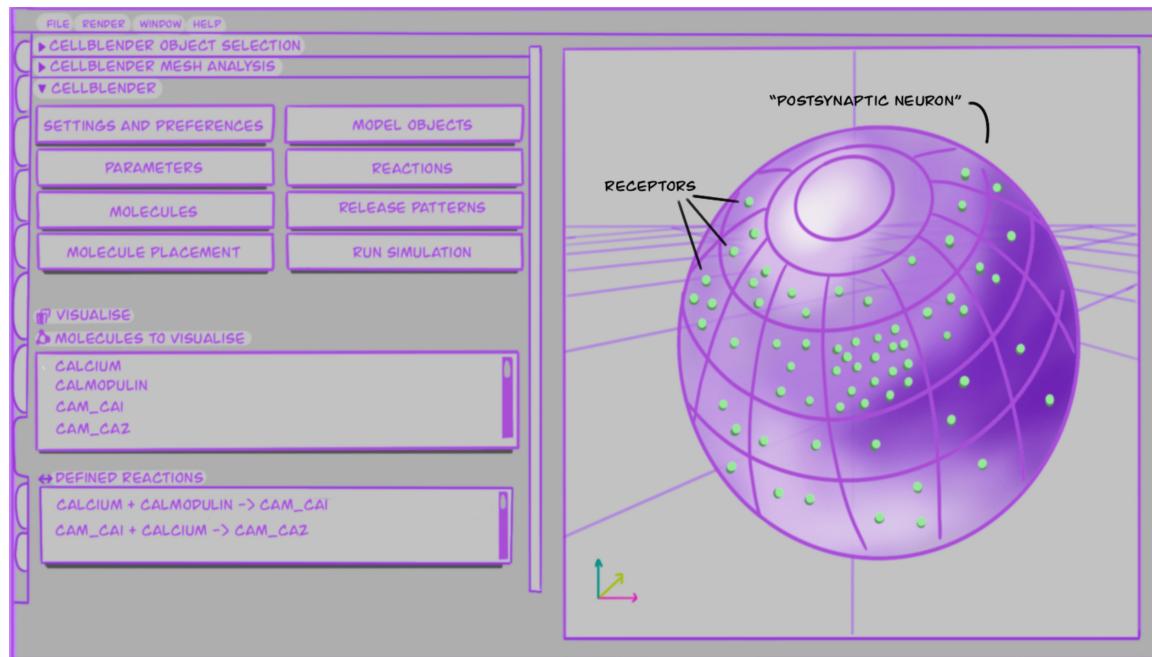


Figure 1.1: A 3D model of a postsynaptic dendritic head, in a schematic of what CellBlender looks like, simplified.

2 Ethics and Reproducibility emphasis in this PhD and why it matters

The work I have carried out through this PhD so far, has had a very heavy focus on thinking about Ethics and Reproducibility as an embedded part of my research, not as a last minute add-on. This is because I truly believe it is of utmost importance that there is a shift in how we proceed with science. A science that currently works under a capitalistic mechanism which advocates for mass production of positivist, fast research that historically mostly only benefits a few (usually cis-male, white, able-bodied individuals¹) ([Webb, Etter, and Kwasa 2022](#)), ([Diogo et al. 2023](#)), ([Branch et al. 2022](#)).

2.1 Science for the profit of whom?

There is a historical heritage of monetary incentivization to move towards drug discovery and the profit that comes from this, and how this has played a key role in biasing research towards drug discovery to “fix” individuals, without their wellbeing being necessarily at the forefront. Dosi et al. ([2023](#)) provide a long term review of Big Pharma and monopoly capitalism, but there are many examples of how companies move scientific research in a way that is driven by economic profit; and how there’s a constant pull to publish more and publish first. It is not the point of this report to go in depth into how or why this has happened, but I can offer you a few good places to start, if you are interested.

The book *Warp and Weft* by Fennan ([2021](#)), with a focus on psychiatry and neuroscience, looks at some of these sciences’ history and examines the ways they have been, and continue to be used as a colonial force. Enforcing a global North science on to the world, as well as describing how many times this enforcement has been led by economic profit for only a few. One good example it cross-references is the “The Mega-Marketing of Depression in Japan” by GlaxoSmithKline, originally spoken about in the book *Crazy Like Us* by Watters ([2010](#)), which is another good place to find examples of this kind.

¹This bias towards benefiting cis-male, white, able-bodied people does not mean they do not suffer, and does not negate the existence of the issues they may experience too. For more information on how society is biased in a way that provides privileges in a certain order/hierarchy, and how to handle it, please have a look at ([DiAngelo 2018](#)), or ([Delgado 2022](#)).

2.1.1 Historical oppressive biases:

It is because of these histories, that I want to attend to them in the work that I do. If we ignore thinking about the ethics, philosophy and history of the research that we do, we may forget where certain ontologies and basis of knowledge come from. Therefore continuing to pretend that these topics are not necessary to think about, whilst a privileged group continues to perpetuate oppressive biases towards historically marginalized groups.

In a presentation I gave in 2022, I give a few examples of biases that continue to happen in science, including examples of racism, sexism, ableism and speciesism ([Garcia, Sterratt, and Stefan 2022](#)). A good example of embedded biases in science is given by Branch et al. ([2022](#)) as they eloquently articulate how a desire to quantify and establish hierarchies among organisms was not purely for scientific interest, but that there is extensive evidence in the fact that the roots of evolutionary biology, which serves as a baseline for many other disciplines like neuroscience, are steeped in histories of white-supremacism, eugenics, and scientific racism. They discuss the definition of the “Not-So-Fit”, and how this limits the diverse thought and investigative potential in biology. This is of importance for my PhD, as I use hierarchies and models of biology that are based on a historical context of how science has reached it’s current status of knowledge.

2.2 Slowing down...

As a response to a fast-paced, profit-driven science, a few Slow Science Manifestos have been published, notably *Another Science is Possible: A Manifesto for Slow Science* by Stengers ([2018](#)) maintains that in order to make higher quality education and science, it needs to serve society as a whole, and calls, among other things, for an “accountability in the knowledge society versus profitability in the knowledge economy”.

Moreover, as long as we continue to create fast research without regard for reproducibility, we will continue to experience what some now call a “Reproducibility Crisis” ([Baker 2016](#)), ([Treves 2022](#)), where we find that, as work is done into trying to reproduce previous published results, this is not possible. The reproducibility or replicability crisis (more on these terms [below](#)) undermines the credibility of theories scientific knowledge; as an essential part of the scientific part of the scientific method is to be able to repeat and reproduce or falsify empirical results and theories.

There is an argument to be made that making research more reproducible and ethical takes more time. And it does. This is precisely why slowing down can help in creating higher quality research that serves all in society.

2.3 Importance of reproducibility

During my PhD work so far, one aspect of the research that has come with quite a lot of struggle is to find accurate parametrization of values for protein dynamics. This is a known issue for most of us who create computational models of biological systems. Wieber and Hocquet (2020) call it an “epistemic opacity” when talking about lack of clarity in Computational Chemistry, where this opacity is entangled in methods and software alike.

This of course leads to reproducibility issues, and as this unfolds, it becomes clear that the “untrustworthiness” of research is also an issue for many other researchers. In fact, a survey of 1576 scientists published in Nature (Baker 2016) reported that over 70% of the participants failed to reproduce others’ experiments and over 50% failed to reproduce their own results.

Interestingly, Tiwari et al. (2021), assessed the reproducibility of 455 mathematical models in systems biology and found that about 50% of published models were not reproducible either due to incorrect or missing information in the manuscript.

Making an effort into creating research that is reproducible can help to avoid wasting resources, including having to repeat the same experiment questions again and again because results from one study could not be reproduced or replicated by other groups.

2.3.1 Defining reproducibility vs replicability

These terms have been used interchangeably for a while, or their meanings being swapped depending on the field of study Claerbout and Karrenbach (1992)], (Ivie and Thain 2018), (Plessner 2018).

Here, we use the definition used by (Turing Way Community et al. 2019), where reproducible research is understood as work that can be independently recreated from the same data and the same code that the original team used. Reproducible is distinct from replicable, robust and generalisable as described in the table below (Figure 2.1).

The different dimensions of reproducible research described in the matrix above have the following definitions, taken from the Turing Way booklet:

- **Reproducible:** A result is reproducible when the *same* analysis steps performed on the *same* dataset consistently produces the *same* answer.
- **Replicable:** A result is replicable when the *same* analysis performed on *different* datasets produces qualitatively similar answers.

		Data	
		Same	Different
Analysis	Same	Reproducible	Replicable
	Different	Robust	Generalisable

Figure 2.1: How the Turing Way defines reproducible research.

- **Robust:** A result is robust when the *same* dataset is subjected to *different* analysis workflows to answer the *same* research question (for example one pipeline written in R and another written in Python) and a qualitatively similar or identical answer is produced. Robust results show that the work is not dependent on the specificities of the programming language chosen to perform the analysis.
- **Generalisable:** Combining replicable and robust findings allow us to form generalisable results. Note that running an analysis on a different software implementation and with a different dataset does not provide generalised results. There will be many more steps to know how well the work applies to all the different aspects of the research question. Generalisation is an important step towards understanding that the result is not dependent on a particular dataset nor a particular version of the analysis pipeline.

2.4 Ethics and Reproducibility go together

Entangled with reproducibility, is thinking about ethics. Because no matter how efficient and reproducible an outcome may be, if it's harming a group of individuals, how good really is this research? Likewise, if a project has taken into account and described potential bias

and harms of their data, but then does not share enough material for their research to be reproduced by others, are we really advancing?

Thinking about reproducibility can in turn help to think how you will share your data, as well as where your own data has come from. Hence, reaching an increased awareness of how your data was sourced and its ethics and potential biases. In order to showcase how I see these topics as being interwoven, I presented a poster titled “Bias and reproducibility in a computational neurobiology PhD’s journey” (Figure 2.2) at the International Conference on Systems Biology (ICSB) in October 2022. On the left side of the poster, I share how to think about the ethics and bias of your research, and on the right side I provide tools for reproducibility. I also wrote about this more in depth in this GitHub repository [here](#).

I created this poster because I wanted to showcase, at a conference full of scientists at different stages of their research, how I take into account bias and reproducibility in my research, and how they could too.

Working with ethics, philosophy, reproducibility and an openness to discuss the wider context of where our research rests, may add a bit of time to the research timeline, but can very much enrich a fuller and more complex understanding of the shortcomings of our research and how to do better moving forward.

Following on the idea that scientists are great at selling the gains in efficiency and accuracy of their work, but less well-practiced in thinking about the ethical implications of our work, I present a framework developed to think about dangers or risks involved with your data and research: Data Hazard Labels, see following Chapter 3.

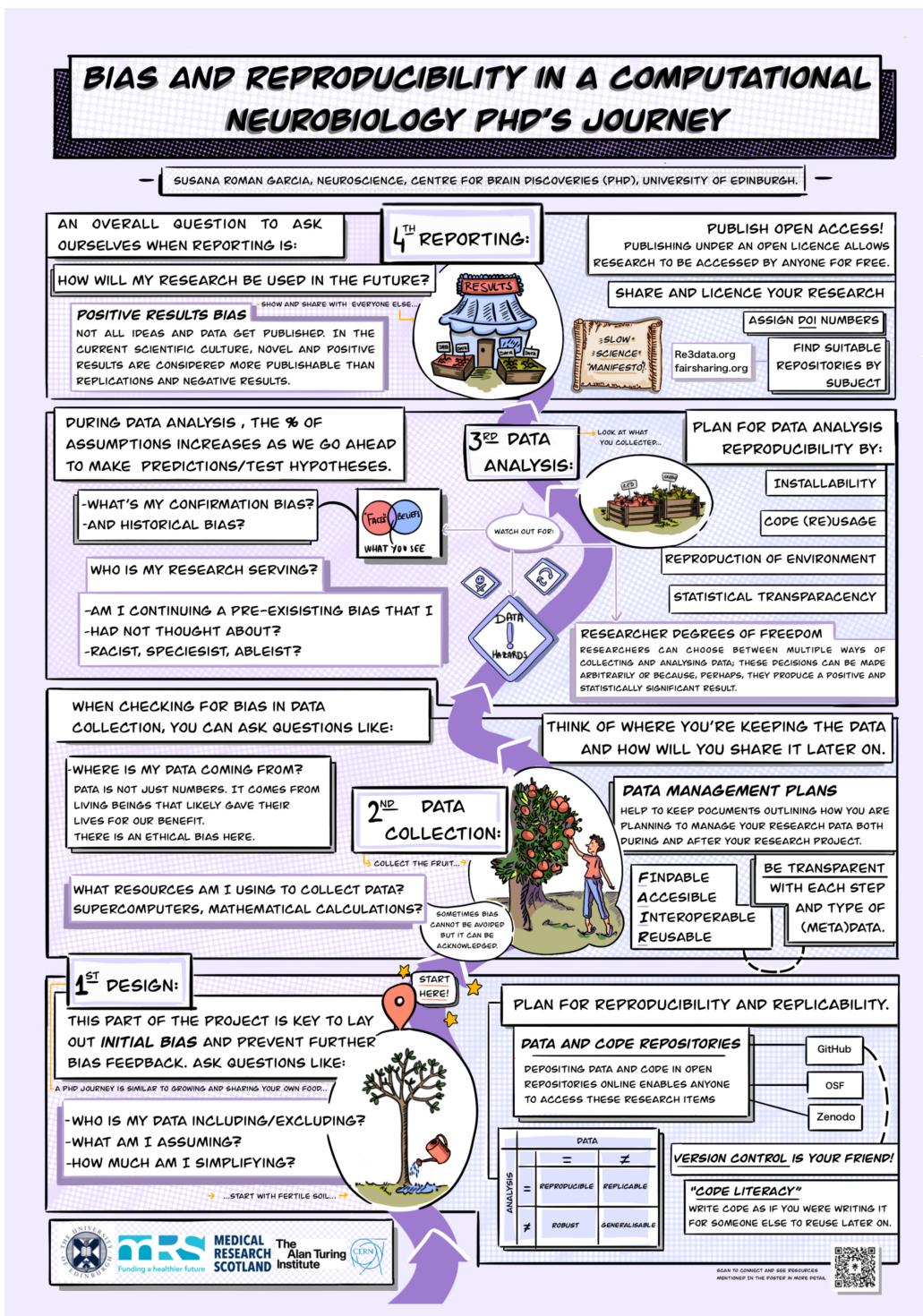


Figure 2.2: Poster about bias and reproducibility, showing research cycle as a journey which starts with design, then data collection, data analysis and final reporting, and compares this through images to growing an apple tree, collecting the apples and then selling them.

3 Data Hazards

The ethical implications that ought to be considered when doing research, usually go beyond what most ethics Institutional Review Boards propose; they should include questions about the wider societal impact of how data science and algorithms work. This is where a project like the [Data Hazards Project](#) comes in handy. Data Hazards is a project made to help us in thinking about worst-case scenarios and ways to mitigate these.

The Data Hazards Project has created a community-developed shared vocabulary of data science risks. The vocabulary presents data ethics concepts in the form of [Data Hazard Labels](#), similarly to chemical hazard labels. This project exists to facilitate material for interdisciplinary discussions and self-reflection about all kinds of data ethics risks. So, how do these labels look like and how can they be implemented, I hear you ask? Let's go through some examples to answer these questions.

3.1 Example label: high environmental cost



Figure 3.1: “High Environmental Impact” Data Hazard Label

3.1.1 Description

This hazard is appropriate where methodologies are energy-hungry, data-hungry (requiring more and more computation), or require special hardware that require rare materials.

3.1.2 Examples

- Example: Running computer models in super computers requires vast energy usage.

3.1.3 Safety Precautions

- Consider in what circumstances it is worthwhile to use this type of methodology.
 - To communicate the scale of the issue to other stakeholders, you may want to convert units of energy into more relatable units.
 - Find out if your cloud provider uses renewable energy.
 - Consider profiling your code, and rewriting it to use less energy.
- Consider future work that would reduce the need to use increasingly more resources.

3.2 How to use the Data Hazards Project

There are four steps to using the Data Hazard labels:

- **Learning:** familiarising yourself with the Data Hazard labels.
- **Applying:** deciding which Hazard labels are relevant to your project.
- **Reflecting:** on what to do differently and what mitigations to make.
- **Display:** displaying the labels alongside your work can help you to communicate that you've thought about these broad ethical issues and how you'd like others to use your work.

This spells LARD , which makes it pretty easy to remember! It is however an unfortunate word it shortens to, as lard comes from dead pigs, so I like to manifest it's a plant-based LARD .

As part of a [Turing Way Book Dash](#) hosted in May 2023, I worked together with a team to create a chapter on Data Hazards for the Turing Way Book. This chapter is still in [draft form](#), but one of the fun things got to do was to work with an artist from Scriberia, to make an illustration of the Data Hazards application (Figure 3.2).

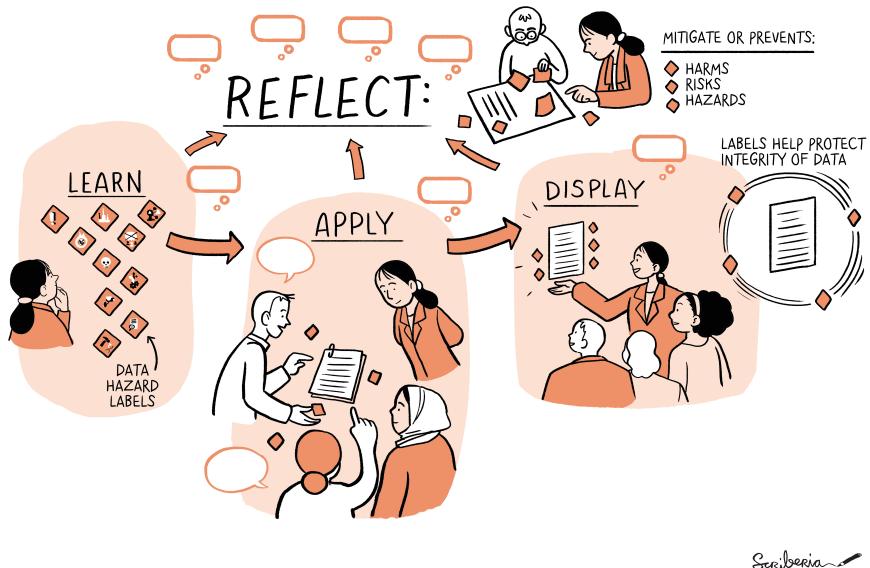


Figure 3.2: Data Hazards Application Cycle ([Community and Scriberia 2023](#)).

3.3 Application example into Research life-cycle

To help visualize where and when Data Hazards can be used in your workflow, below is an example assuming four main stages of workflow: design, data collection, data analysis and reporting. This is a generalised example, but something like this is what it looks like for me when I work on my PhD.

3.3.1 Design:

- Are you using data? Then doing some reflection on [identity and positionality](#) could help you think of what Data Hazards labels you might encounter as you design your project, for example “[ranks of classifies people hazard](#)” or “[risk to privacy](#)” could apply at this stage.
- In this part of the workflow, you might want to prepare to avoid certain Data Hazards if you can, and if you can’t avoid them because of where your data has come from, you may want to acknowledge this. For example, if you a [sensitive data project](#), what Data Hazard labels will apply, and/or what can you do to design your project in a way that avoids certain harms?

3.3.2 Data Collection and Analysis:

- As you are collecting and analyzing your data, you might want to iteratively think of the potential Data Hazards that exist in the information you are collecting. To then apply the labels as you perform the next step of the process: reporting.

3.3.3 Reporting:

- When reporting your results, you can think of applying and reporting the Data Hazard labels that are relevant for your project; examples of how others have done this can be found here [link to self reflection and case studie(s)]. Labeling your project with Data Hazards should also include considerations of mitigations to these risks. This would then be helpful for people who see your outputs in the future. They can be aware of potential risks as they proceed with the project, and continue to think of solutions to any issues related to the research topic.

3.4 Application into my PhD project: Presenting my PhD as a case study at AI UK conference

In order to showcase how Data Hazards can be reflected upon during a PhD, and taking the self-reflection described above into consideration, I have been implementing thinking about the vocabulary they provide into my own work. In line with this work, I made a poster that summarised aims of my PhD, for people to be able to say which labels they thought applied to my project. This poster was part of an exhibition stand with the Data Hazards Team, at [AI UK 2023](#). When creating this poster (Figure 3.3), I was able to both do some self-reflection and collaborative reflection, as described below.

- **Self-reflection (*what is my project and how will it be used?*):**

When making the poster, this kind of self-reflection questions are useful for oneself to think about, but also for external people who are not involved with your project to understand what potential data hazards it might have. The final poster can be seen below in (Figure 3.3). I followed the prompt questions available in the Data Hazards website for project owners who would like their projects to be discussed for data hazards:

- The overall objective of the project.
- Fairly detailed description of the variables in the dataset they are using (and what is not included).

- How and when the data was collected.
- Any statistical/algorithmic methods being used.
- Who has input on the project.
- What outputs are expected, and how these will be shared.

- **Collaborative reflection (*what data hazards may apply to my project?*):**

Then, during the poster presentation, people would come over and talk about the project, have a look at the poster, and decide by adding stickers to a list of hazards, to say which ones applied to it. You can see below how it looked (Figure 3.4).

So, as can be seen in the photo of me happily posing next to the poster Figure 3.4 (before end of the day), people were adding stickers to record which data hazard labels they thought applied to my PhD project. At the end of the day, I recorded final numbers and the results can be seen in the barchart below Figure 3.5.

3.4.1 Results from collaborative reflections:

- **Difficult to understand Label was chosen the most** Interestingly, not all labels were chosen as applicable to my project (Figure 3.5). Only 6 of the 11 current labels were chosen as relevant, with “difficult to understand” being the most prevalent one, chosen by 6 people. High environmental impact and danger of misuse follow in closely with 5 people having chosen these ones. Of course these numbers are small and hold, more than anything, illustrative value as to how and why people may think certain labels apply to a project. In the case of my PhD project, which involves understanding of very specific molecules, as well as knowledge of programming and computer modelling software, it makes sense that the “difficult to understand” label was the one people chose the most.
- find and explain mitigations for these hazards. show examples of how im thinking to mitigate them.

MAKE A TABLE OF DATA HAZARDS -md or html or python?

Table 3.1: Caption

Data Hazard Description	Safety Precautions
 <p>Difficult to understand. There is a danger that the technology is difficult to understand. This could be because of the technology itself is hard to interpret (e.g. neural nets), or problems with it's implementation (i.e. code is not provided, or not documented). Depending on the circumstances of its use, this could mean that incorrect results are hard to identify, or that the technology is inaccessible to people (difficult to implement or use).</p>	<ul style="list-style-type: none"> • Make research code Open Source with an appropriate software license where possible. Your local Research Software Engineering group may be able to help you with this. • Compare results to white box (explainable) methods such as Random Forest or Regression, which may perform just as well. • Ensure code is well documented with accompanying and/or inline documentation.

Data Hazard Description	Safety Precautions
 <p>High environmental impact. There is a danger that the technology is difficult to understand. This could be because of the technology itself is hard to interpret (e.g. neural nets), or problems with it's implementation (i.e. code is not provided, or not documented). Depending on the circumstances of its use, this could mean that incorrect results are hard to identify, or that the technology is inaccessible to people (difficult to implement or use).</p>	<ul style="list-style-type: none"> • Make research code Open Source with an appropriate software license where possible. Your local Research Software Engineering group may be able to help you with this. • Compare results to white box (explainable) methods such as Random Forest or Regression, which may perform just as well. • Ensure code is well documented with accompanying and/or inline documentation.

Data Hazard Description	Safety Precautions
 <p>Danger of misuse. There is a danger that the technology is difficult to understand. This could be because of the technology itself is hard to interpret (e.g. neural nets), or problems with it's implementation (i.e. code is not provided, or not documented). Depending on the circumstances of its use, this could mean that incorrect results are hard to identify, or that the technology is inaccessible to people (difficult to implement or use).</p>	<ul style="list-style-type: none"> • Make research code Open Source with an appropriate software license where possible. Your local Research Software Engineering group may be able to help you with this. • Compare results to white box (explainable) methods such as Random Forest or Regression, which may perform just as well. • Ensure code is well documented with accompanying and/or inline documentation.

blablabla where does text and table render in pdf? text to test this

3.5 Data Hazards Workshops

- Talk about application of DH into different workshop setups, in COMBINE and Turing Institute.
- Most info available here: https://github.com/Susana465/Data_Hazards_workshops, re-write.

MODELS OF CAMKII/NMDAR INTERACTIONS IN THE POSTSYNAPTIC NEURON

- PROJECT DESCRIPTION:-

I CREATE 3D MODELS WHICH SIMULATE INTERACTIONS BETWEEN PROTEINS IMPORTANT FOR UNDERSTANDING HOW MEMORY WORKS IN ANIMAL BRAINS.

- AIM & SIGNIFICANCE:-

EXPLAIN HOW SPECIFIC MOLECULES WORK TOGETHER DURING MEMORY.

DEVELOP NEW WAYS OF 3D MODELLING TO LOOK AT COMPLEX PROCESSES IN NEURONS.

THE MOLECULES I LOOK AT HAVE BEEN SHOWN TO BE DYSFUNCTIONAL IN ALZHEIMER'S AND HUNTINGTON'S DISEASE.

- TYPE OF DATA:-

KINETIC RATES OF MOLECULE REACTIONS, MOLECULAR CONCENTRATIONS COLLECTED FROM LITERATURE AND DATABASES.

NUMBERS OBTAINED FROM EITHER WET LAB EXPERIMENTS OR MATHEMATICALLY CALCULATED.

- METHODS:-

MODELS WRITTEN WITH STANDARDISED, OPEN SOURCE MODEL LANGUAGES (PYTHON, BNGL).

SIMULATIONS RUN USING FREE, OPEN SOURCE MODELLING TOOLS.

RUN LOCALLY OR IN CLUSTER FOR 3+ HOURS IF SIMULATIONS ARE MORE COMPUTATIONALLY EXPENSIVE.

- MODEL APPLICATIONS:-

OTHER RESEARCHERS CAN BUILD FROM THESE MODELS TO CREATE FURTHER PREDICTIONS FOR POTENTIAL PHARMACOLOGICAL APPLICATIONS.

WHICH DATA HAZARDS APPLY? COMPLETE POLL HERE!

SUSANA ROMAN GARCIA, PhD STUDENT,
UNIVERSITY OF EDINBURGH

Figure 3.3: PhD Project description - Case Study, to see GitHub repo, click on this figure.



Figure 3.4: Data Hazards Case Study Poster at AI UK

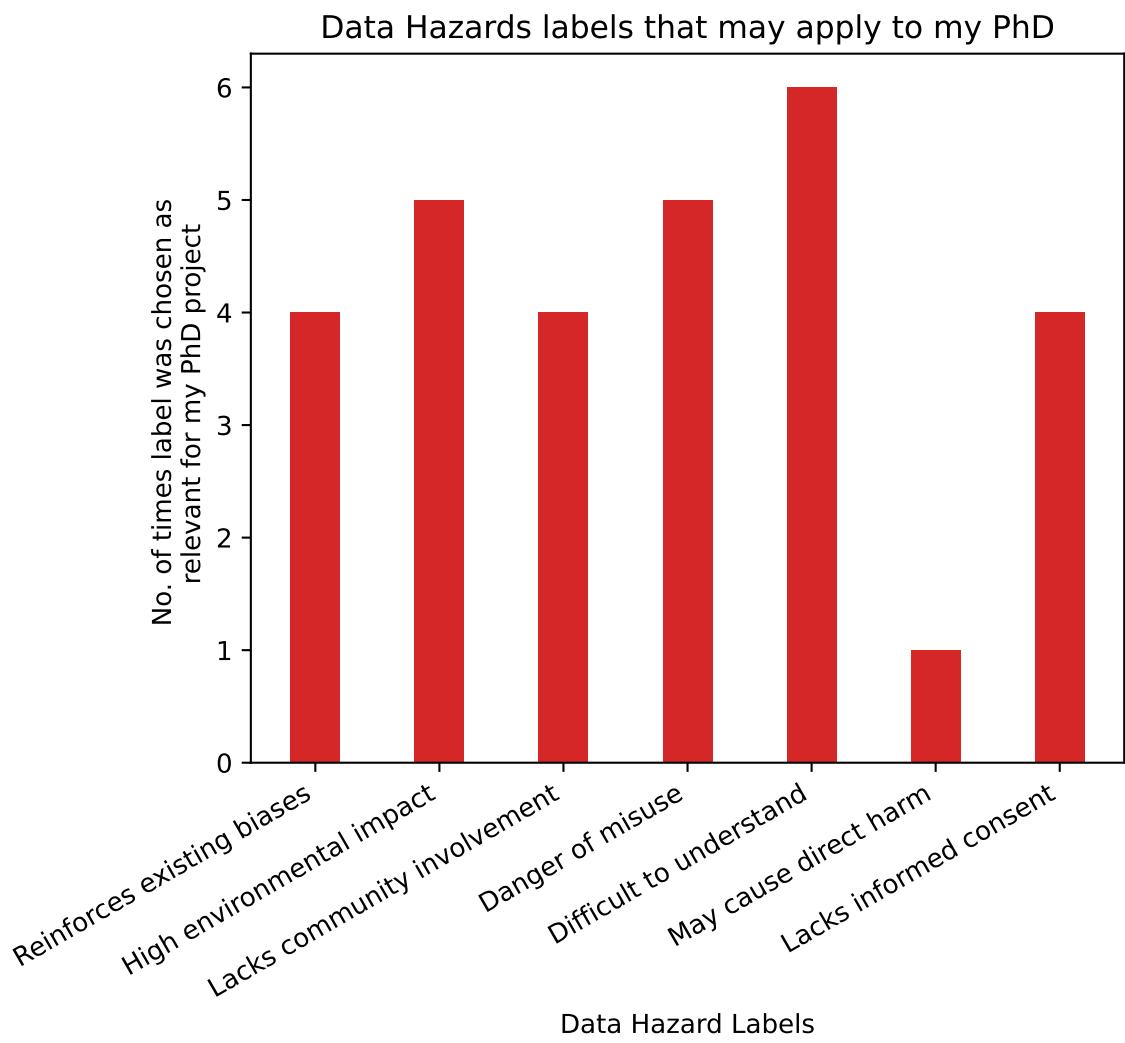


Figure 3.5: Data Hazards labels that may apply to my PhD.

4 Computer models of CaMKII/NMDAR interactions

4.1 Software used: BioNetGen and MCell

4.1.1 BioNetGen and how rule-based modelling can help with combinatorial complexity.

BioNetGen is a set of software tools which facilitate a rule-based approach to modelling biochemical reaction kinetics, where we can largely overcome the problem of combinatorial complexity that arises when modelling CaMKII. It has been calculated that CaMKII as a dodecamer can approximately have 1020 possible states ([Pharris et al. 2019](#)); this, coupled with the potential of a full reaction network for each simulation (an added factor of combinatorial complexity), can render the model computationally intractable. BioNetGen can help us deal with this combinatorial complexity thanks to its rule-based modelling (RBM) “don’t care, don’t write” capabilities.

BioNetGen language (BNGL) is a formal language which uses the BioNetGen software ([Faeder, Blinov, and Hlavacek 2009](#)). It allows for site-specific details of protein-protein interactions to be captured in models for the dynamics of these interactions in a systematic fashion, which also alleviates nomenclature and reusability issues.

Hence, using this RBM approach is notable as it facilitates writing of multi-state modelling and can significantly, reduce the number of reactions that need to be written due to its “don’t write, don’t care” characteristic. Thereby dramatically improving the ability to model CaMKII as a dodecamer; I can make a model with multistate molecules, and specify the states of the reactants that are relevant for a particular reaction, and leave the rest unspecified.

```
# BioNetGen code which shows a simple model of A, B,C molecules \
that is helpful for understanding the "don't care, don't write" \
capacity of rule based modelling.
```

```
begin model
```

```

begin parameters
# Define initial number of molecules released
A_i 150
B_i 150
C_i 100

#Define reaction rates
kon 1e-2
koff 1e-3
k_P 1e1
end parameters

begin molecule types
# Define the molecules and the possible states and \
binding sites they can have:

# Molecule A has a binding site (a), and a Phosphorylation site \
which can be unphosphorylated (~0) or phosphorylated (~P)
A(a,T286~0~P)
# Molecule B has a binding site (b)
B(b)
# Molecule C has no binding sites
C()
end molecule types

begin species
# Molecule A starts with binding site a free, \
and with phosphorylation site unphosphorylated
A(a,T286~0) A_i
# Molecule B starts with binding site b free
B(b) B_i
# Molecule C has no binding sites so it starts as it is
C() C_i
end species

begin reaction rules
# A_free and B_free can reversibly bind to give AB_complex
# Don't need to specify, if I'm not interested, status of \
phosphorylation for molecule A. Note how it is not written \
in the rule definition (don't care, don't write)

```

```

A(a) + B(b) <-> A(a!1).B(b!1) kon, koff

# If A is unphosphorylated, it can become phosphorylated \
by the presence of C. Don't need to specify status of \
binding site 'a' (don't care, don't write)
A(T286~0) + C() -> A(T286~P) k_P

end reaction rules

begin observables
Molecules AB_complex A(a!1).B(b!1)
Molecules A_phosphorylated A(T286~P)
Molecules A A(a)
Molecules B B(b)
Molecules C C()
end observables

end model
simulate({method=>"ssa",t_end=>10,n_steps=>100})

```

4.1.2 MCell (Monte Carlo Cell) and how it simulates reactions in 3D

Is a biochemistry simulation tool that uses spatially realistic 3D cellular models and stochastic Monte Carlo algorithms to simulate the movements and interactions of discrete molecules within and between cells, ([Bartol and Stiles 2000](#)), ([Kerr et al. 2008](#)), ([Bartol et al. 2015](#)). MCell is a particle-based simulator that represents molecules as point particles in 3D space. At every time step in an MCell simulation, each particle can move, collide with other particles or surfaces, and undergo bimolecular and unimolecular reactions. The basic elements of a simulation step are as seen in Figure 4.1 taken from Gupta et al. ([2018](#)).

Briefly, MCell operates as follows: as a volume molecule diffuses, all molecules within a given radius along its trajectory, or at the point of collision on a surface, are considered for a reaction. For surface molecules (in membranes), the molecule first diffuses, and then its neighbours are evaluated for reaction.

There is no volume exclusion for molecules diffusing in 3D volumes, and molecules on surfaces occupy a fixed area. MCell allows defining arbitrary geometry Figure 4.1 (C), and complex models such as a 180 m³ 3DEM reconstruction of hippocampal neuropil have been used to construct a geometrically-precise simulation of 100s of neuronal synapses at once ([Bartol et al. 2015](#)). A detailed description of mathematical foundations of MCell's

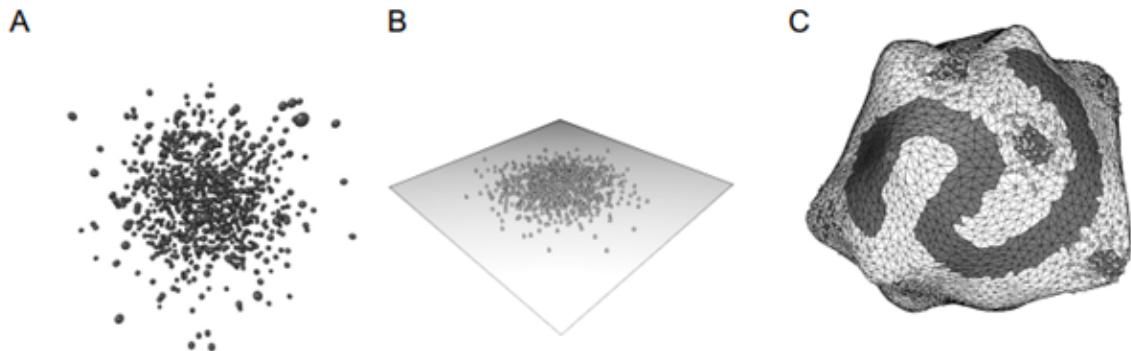


Figure 4.1: MCell Components. (A) Volume Molecules diffusing in free space. (B) Mesh Object defined by a Plane with Surface Molecules diffusing on it. (C) Mesh Object defined by a complex closed mesh with multiple defined Surface Regions, in which Surface Molecules have different diffusion constants, as defined by corresponding Surface Classes.

algorithms can be found here: Bartol and Stiles (2000), Kerr et al. (2008), Bartol et al. (2015).

MCell4, version used for this project, provides a versatile Python interface, which is very useful for writing models with said interface and running mcell models this way. MCell 4 provides two different user experiences, one through its visual interface as an add-on in Blender 2.93, known as CellBlender (see back at Figure 1.1), the other user experience one through a new Python interface. This provides users with the flexibility to change between both experiences, or to run the simulations using Python and visualize the simulations in Blender.

Testing to see if I can make a visual diagram of how this works.



4.2 Model description

(from last years report, needs updating) I have constructed the models at different scales to validate CaMKII interactions with other molecules like calmodulin and NMDARs, at increasing levels of complexity. First I re-created a model of CaMKII as a monomer that was previously completed in 2017. The model created uses cBNGL and represents CaMKII

as monomers to serve as a proof of concept as well as a starting validation point, as dynamics of this model were previously shown to be within biologically accurate limits. Secondly, I created a model of CaMKII as a hexamer since modelling this molecule as a dodecamer gave rise to a combinatorial explosion due to the high number of possible states and the network of interactions generated. This is currently in the works of being resolved as I run the model using the network-free simulation using MCell. We will then be able to model CaMKII as a dodecamer. Finally, I aim to validate this work against a model from Ordyan et al., 2020, where they successfully modelled CaMKII as a twelve subunit holoenzyme using BioNetGen simulations.

As explained in the introduction of this report, CaMKII is a dodecameric molecule, meaning it's composed of twelve subunits. Ideally, I would like to model CaMKII as a dodecamer since this would allow us to infer more accurately any emergent behaviour of the protein. However, due to combinatorial explosion, running a CaMKII as a dodecamer takes a really long time: 6+ hours without finalising run time, using a cBNG, SSA simulation. These simulations include only calcium binding to CaM, and CaM binding to CaMKII as a dodecamer, without further reactions added to avoid further complexity. The machine being used for these simulations is a x64-based laptop, with specs: 11th Gen Intel® Core™ i5-1145G7@2.60GHz, 1498Mhz, 4Core(s), 8 Logical Processors. SSA simulations generate a full network of reactions and causes the simulation to slow down considerably. At the moment, compartmental BNGL does not allow for NFsim simulations to be ran. This can be overcome by running the model with MCell as it provides a network free simulation capability. An alternative so far has also been to run the model with CaMKII as a hexamer. However, it still causes combinatorial explosion when generating network reactions. This should be resolved by running the model as a network free simulation in MCell.

4.2.1 Model of CaMKII as a dodecamer

4.3 Model development and validation

Following from what I did last year, show results, copy what's on github <https://github.com/Susana465/CaMKI>

Develop the description of how the models work – model description and results. Biologist friendly description of the model.

talk about robustness, generalisable, environments

Write abstract for each chapter, then merge them altogether.

4.3.1 A reproducible model

The same processes used in software development can also be applied to biological model development. Therefore, when developing the models in this project, four main points were considered throughout, as suggested by Husar et al. (2022):

1. Create incremental development where the model is built step by step, relying on solid foundations of modelling done and validated before,
2. Create a modularity that provides the capability to create self-contained, reusable libraries,
3. Perform unit testing and validation to verify that parts of the model behave as expected and,
4. Create human-readable and writable model code that can be stored using git or other code version control software which also allows code reviews so that other team members can inspect the latest changes to the model.

5 Activities

- what have i been up to?

6 Thesis layout

1.1. Report style and philosophy 3

- 1.1.1. Unusual stylistic choices in this report 3
- 1.2. Complexity Science and Systems Biology 3
- 1.3. Team-efforts Science 4
- 1.4. Open and Reproducible Science 4
- 1.5. Synapses and neuronal plasticity: a brief background (for beginners) 8
- 1.6. Memory and learning: 9
- 1.6.1. Why study CaMKII and NMDAR interactions to study memory formation? 9
- 1.6.2. A brief history background on learning and memory research: 9
- 1.6.3. Long Term Potentiation 9
- 1.6.4. NMDA receptors structure and functions 10
- 1.6.5. CaMKII structure and functions 11
- 1.6.6. Bringing it all together: LTP, CaMKII/NMDAR complex as a molecular memory and interactions within the postsynaptic neuron 13
- 1.7. Why use Computational Modelling to study biological systems? 14
- 1.7.1. How do we model biochemical systems networks? 16
- 1.8. Why are we using Rule-Based Modelling to model CaMKII interactions? 21
- 1.8.1. CaMKII combinatorial explosion: how do we resolve this? 22
- 1.9. MCell and how it simulates reactions in 3D 23
- 1.10. Biodynamo 25
- 2. METHODS 25
- 2.1. Model Validation 25
- 2.2. Model description 25
- 2.2.1. Model of CaMKII monomers 26
- 2.2.2. Model of CaMKII hexamers 26
- 2.2.3. Model Creation and Visualization in CellBlender 26
- 2.3. Creating accessible and reproducible research 29
- 2.3.1. Learning to use Jupyter Notebook 29
- 3. RESULTS 30
- 3.1. Results from CaMKII monomer model 30
- 3.2. Hexamer CaMKII interactions with calmodulin and phosphorylation behaviour. Does our model reproduce previously suggested results of CaMKII activation? 31
- 4. Work envisaged for the remainder of the project 35
- References 37

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