NUFFIELD RESEARCH PROJECT

Computational Modelling of FMRP Regulation

Lewis Strachan
Cardinal Newman High School

Mr Richard Fitzpatrick
Dr Melanie Stefan

Centre for Integrative Physiology
The University of Edinburgh

THE UNIVERSITY of EDINBURGH

Nuffield Foundation

Abstract
Fragile X Syndrome is a developmental disorder caused by the lack of the Fragile X Mental Retardation Protein (FMRP). In this investigation, we created a computational model of this protein composed of the biochemical reactions it and its related compounds are involved in. Here, we report that FMRP synthesis is the rate-limiting step of the biochemical system and that its regulation in turns regulates the system. We also report that receptor binding events play a large role in the regulation of the CYFIP1-eIF4E-FMRP complex (CEF) which is the form that FMRP takes when involved in translational repression.

Introduction
In my Nuffield Research Placement I was tasked with creating a computational model of FMRP regulation. FMRP (Fragile X Mental Retardation Protein) is a protein normally expressed in humans and model organisms (e.g. rats, mice) but we are particularly interested in its function in the brain. FMRP has been proven to be a translational repressor (Laggerbauer, 2001) which means that it prevents other proteins being made and this has been shown to be necessary to prevent excess protein synthesis (See Appendix 1). Unfortunately, there is a neurodevelopmental disorder which is caused by a lack of FMRP: Fragile X Syndrome.

Fragile X Syndrome (FXS):
FXS is the most common form of inherited intellectual disability and is also the most common cause of Autism Spectrum Disorders. Symptoms of FXS include: ADHD (Attention Deficit Hyperactivity Disorder), anxiety, sensory hypersensitivity, unstable mood, and seizures. Sufferers also display physical abnormalities such as a long face, prominent ears, flat feet, and hyperextensible joints (Bagni et al., 2012). The intellectual disability aspect of the condition varies greatly between males and females. Both sexes experience severe learning disabilities, especially when learning to speak. Male sufferers of the condition have an average IQ of about 40 and show problems with short-term memory. Female sufferers are less affected because they have two X chromosomes and only one is active in each cell so there is a greater production of FMRP. About half of female sufferers have IQs in the borderline or mild intellectual disability range. (Intellectualdisability.info, 2017)

FXS is a trinucleotide repeat expansion disorder which means it is caused by a certain type of mutation in the gene that codes for FMRP. Genes are composed of a sequence of bases (Adenine, Guanine, Cytosine, and Thymine; represented by the letters A, G, C, and T respectively) and each group of three bases (known as a triplet) codes for an amino acid and these then join together to form proteins. In non-sufferers of FXS their FMRP genes have about 5-44 repeats of the CGG triplet (Bagni et al., 2012)

which is normal and harmless. Sufferers of FXS have greater than 200 repeats of the CGG triplet (Bagni et al., 2012) which causes the gene to be methylated (silenced) and no FMRP is made. There is also a related condition caused by the premutation of FXS called Fragile X – Associated Tremor/Ataxia Syndrome (FXTAS) which is when the CGG triplet occurs between 55 and 200 repeats (Bagni et al., 2012) (See Appendix 2).

FXS is a genetic disorder and as a result is hereditary. The gene is located on the X chromosome and so the condition follows a sex-linked pattern of inheritance. This means that the condition is generally more prominent in males than females; it is estimated that FXS affects "approximately 1 in 2,500 to 5,000 men and 1 in 4,000 to 6,000 women". (Bagni et al., 2012). This is due to the fact that males only need one mutated allele (copy of the gene) as they only have one X chromosome, but women have two so need two mutated alleles to have the condition.

Biochemical Modelling:

A biochemical model is composed of a series of reactions (a "system") which have reaction rates (kf/kb values) and these reactions are composed of species (not related to living organisms, these species are proteins, molecules etc.) which have concentrations. The rates give the speed at which reactions occur, kf for the forward rate, kb for the reverse reaction. When a model is run, the reactions take place and the concentrations of the species as time progresses are plotted on graphs. My biochemical model (visualised in Appendix 3) was generated by collecting data from scientific literature and other similar models. The reactions in my model can be divided into the following subsections.

Biochemical modelling is an incredibly useful tool for many reasons. Firstly, they allow us to avoid animal experimentation by using current data to run a series of reactions on a computer instead of inside an animal, thereby being more ethically acceptable. Modelling allows parameters to be changed that would be impossible to change in a living animal/cell and allows these parameters to be tested quickly without having to perform many replicates to obtain reliable data experimentally.

FMRP and FXRP1/FXRP2 Heterodimer Association:

FMRP forms heterodimers (Bimolecular compounds composed of different molecules) with FXRP1 and FXRP2, which are homologues of FMRP and share many of the same binding targets due to their similar structure. The functions of FXRP1 and FXRP2 are currently unknown (Dolzhanskaya, 2006).

FMRP Regulation:

FMRP is synthesised by the expression of the FMR1 gene. It is then quickly phosphorylated (addition of a phosphate group) by the enzyme S6K (S6 Kinase) and becomes active, i.e. it takes part in reactions and acts as a translational repressor (Narayanan et al., 2008). This phosphorylated variant of FMRP can then be dephosphorylated by the enzyme PP2A (Protein Phosphatase 2A) and is inactivated, i.e. it no longer participates in reactions and does not act as a translational repressor (Narayanan et al., 2007). This cycle of phosphorylation/ dephosphorylation can repeat but is affected by various factors. For example, the binding of Glutamate to mGluR (Metabotropic Glutamate Receptor) increases PP2A activity (Mao et al., 2005) and the binding of BDNF (Brain-Derived Neurotrophic Factor) to TrkB (Tropomyosin Receptor Kinase B) increases S6K activity (Lenz and Avruch, 2005). The dephosphorylated variant of FMRP can be tagged with a small protein called Ubiquitin in a series of enzymatic reactions. When tagged, the FMRP is transferred to a proteasome, which is composed of numerous proteases (enzymes that break down proteins), where it is degraded into its constituent amino acids (Nalavadi et al., 2012).

CEF Formation:

The CEF complex (CYFIP1-eIF4E-FMRP complex) is a heterotrimer that inhibits translation. It is composed of FMRP, CYFIP1 (Cytoplasmic FMRP-Interacting Protein 1), and eIF4E (Eukaryotic Translation Initiation Factor 4E). CEF can be formed in multiple ways. Firstly, any two of the three complex molecules bind to form a heterodimer, and secondly the remaining complex molecule binds to the heterodimer to form the CEF heterotrimer (Napoli et al., 2008; De Rubeis et al., 2013). Of particular note, the binding of Glutamate to mGluRs and of BDNF to TrkBs accelerates the breakdown of the CYFIP1-eIF4E heterodimer (De Rubeis et al.,2013).

Hypotheses

The main aim of the investigation being to generate the computational model. However, we had some small hypotheses that we wished to test:

- FMRP is centrally important to the functioning of the system
- The regulation of FMRP in turn regulates the system
- Receptor binding events play a large role in the regulation of the system

Method

The computational model was developed with COPASI (Complex Pathway Simulator) using data from scientific literature and other similar models. COPASI is a "software application for simulation and analysis of biochemical networks and their

dynamics" (Copasi.org, 2017) and is mainly used as a deterministic simulator, which means that if you run the model with the same variables several times the results will be the same (See Appendix 4 for COPASI UI). This is in contrast to spatial modelling software (e.g. CellBlender) that take factors like collision geometry, activation energy and the position of species into account.

The input data for the model can be seen in Figures 1 to 3.

The compartment volume was set to 9e-17l (Hayer and Bhalla, 2005) as this is the volume of dendritic spine heads which are the locations of the reactions in my model.

Figure 1 – Species (Adjust if necessary)

Species	**Initial Concentration (µmol/l)**	**Source**
P-FMRP	0.4	Estimate
FXRP1	0.4	Estimate
FXRP2	0.4	Estimate
FMRP-FXRP1	0	-
FMRP-FXRP2	0	-
CYFIP1	0.6	Estimate
eIF4E	0.4	Rau et al., 1996
CYFIP1-FMRP	0	-
CYFIP1-eIF4E	0	-
FMRP-eIF4E	0	-
CEF	0	-
mGluR	0.3	DOQCS (Database of Quantitative Cell Signalling)
Glu	*	Fitzpatrick, 2017
mGluR-Glu	0	-
TrkB	0.25	DOQCS
BDNF	*	Fitzpatrick, 2017
TrkB-BDNF	0	-
DP-FMRP	0	-

Species	Initial Concentration (µmol/l)	Source
U-FMRP	0	-
PP2A	1.11	Fitzpatrick, 2017
S6K	1.25	DOQCS
UE	0.12	Estimate
null	1 (Fixed)	-

* Glu Expression: if({Values[Time]} >= 100,0,if({Values[Time]} >= 50,1,0))
* BDNF Expression: if({Values[Time]} > 300,0.1,if({Values[Time]} > 200,3.7,0.1))

Figure 2 – Reactions

Reaction	Equation	Rate	Source
FMRP-FXRP1 (forward)	P-FMRP + FXRP1 → FMRP-FXRP1	49.999l/(µmol*s)	DOQCS
FMRP-FXRP1 (backward)	FMRP-FXRP1 → P-FMRP + FXRP1	0.15s^-1	DOQCS
FMRP-FXRP2 (forward)	P-FMRP + FXRP2 → FMRP-FXRP2	49.999l/(µmol*s)	DOQCS
FMRP-FXRP2 (backward)	FMRP-FXRP2 → P-FMRP + FXRP2	0.15s^-1	DOQCS
CYFIP1-FMRP (forward)	P-FMRP + CYFIP1 → CYFIP1-FMRP	49.999l/(µmol*s)	DOQCS
CYFIP1-FMRP (backward)	CYFIP1-FMRP → P-FMRP + CYFIP1	0.15s^-1	DOQCS
CYFIP1-eIF4E (forward)	CYFIP1 + eIF4E → CYFIP1-eIF4E	49.999l/(µmol*s)	DOQCS
CYFIP1-eIF4E (backward)	CYFIP1-eIF4E → CYFIP1 + eIF4E	0.15s^-1	DOQCS
FMRP-eIF4E (forward)	P-FMRP + eIF4E → FMRP-eIF4E	49.999l/(µmol*s)	DOQCS
FMRP-eIF4E (backward)	FMRP-eIF4E → P-FMRP + eIF4E	0.15s^-1	DOQCS

Reaction	Equation	Rate	Source
CEF1 (forward)	CYFIP1-eIF4E + P-FMRP → CEF	49.999l/(μmol*s)	DOQCS
CEF1 (backward)	CEF → CYFIP1-eIF4E + P-FMRP	0.15s^-1	DOQCS
CEF2 (forward)	CYFIP1-FMRP + eIF4E → CEF	49.999l/(μmol*s)	DOQCS
CEF2(backward)	CEF → CYFIP1-FMRP + eIF4E	0.15s^-1	DOQCS
CEF3 (forward)	FMRP-eIF4E + CYFIP1 → CEF	49.999l/(μmol*s)	DOQCS
CEF3 (background)	CEF → FMRP-eIF4E + CYFIP1	0.15s^-1	DOQCS
mGluR-Glu (forward)	mGluR + Glu → mGluR-Glu	16.8l/(μmol*s)	Fitzpatrick, 2017
mGluR-Glu (backward)	mGluR-Glu → mGluR + Glu	10s^-1	Fitzpatrick, 2017
TrkB-BDNF (forward)	TrkB + BDNF → TrkB-BDNF	0.99998l/(μmol*s)	Fitzpatrick, 2017
TrkB-BDNF (backward)	TrkB-BDNF → TrkB + BDNF	0.0499967 s^-1	Fitzpatrick, 2017
mGluR-Glu CYFIP1-eIF4E Dissociation	CYFIP1-eIF4E → CYFIP1 + eIF4E; mGluR-Glu	kcat = 75s^-1 KM = 40 μmol/l	Estimate
TrkB-BDNF CYFIP1-eIF4E Dissociation	CYFIP1-eIF4E → CYFIP1 + eIF4E; TrkB-BDNF	kcat = 75s^-1 KM = 40 μmol/l	Estimate
P-FMRP Dephosphorylation	P-FMRP + PP2A → DP-FMRP + PP2A	PP2Arate	Fitzpatrick, 2017
DP-FMRP Phosphorylation	DP-FMRP + S6K → P-FMRP + S6K	S6Krate	Fitzpatrick, 2017
DP-FMRP Ubiquitination	DP-FMRP + UE → U-FMRP + UE	0.5l/(μmol*s)	Estimate
U-FMRP Degradation	U-FMRP → null	1s^-1	Estimate

Reaction	Equation	Rate	Source
FMRP Synthesis	null → P-FMRP	0.001s^-1	Estimate

Figure 3 – Global Quantities

Global Quantity	Unit	Expression
Time	s	{Time}
PP2Arate	l/(μmol*s)	if({[mGluR-Glu]} > 0.1,12,6)
S6Krate	l/(μmol*s)	if({[TrkB-BDNF]} > 0.2,0.02,0.01)

The model was then run with varied concentrations and reaction rates to investigate specific factors on the biochemical system. The model was run using the Time Course and Parameter Scan tasks. The Time Course task runs the model for a set duration and plots the concentrations of the species over time. The Parameter Scan task is similar to a Time Course but one value (concentration or reaction rate) is varied through a range and this can be used to investigate the effects of the variable on the system. Parameter scans were used to verify if a species' concentration had a significant impact on the system so we could make estimates for species that we did not know their concentration from current data.

Results

The following graphs all display the changes in various species over time. Time is represented on the x-axis and is measured in seconds, and species concentration is represented on the y-axis and is measured in μmol/l (micromoles per litre). Each line represents the concentration of a species over time. The scale on the y-axis is scaled depending on the values displayed on the graph.

Figure 4

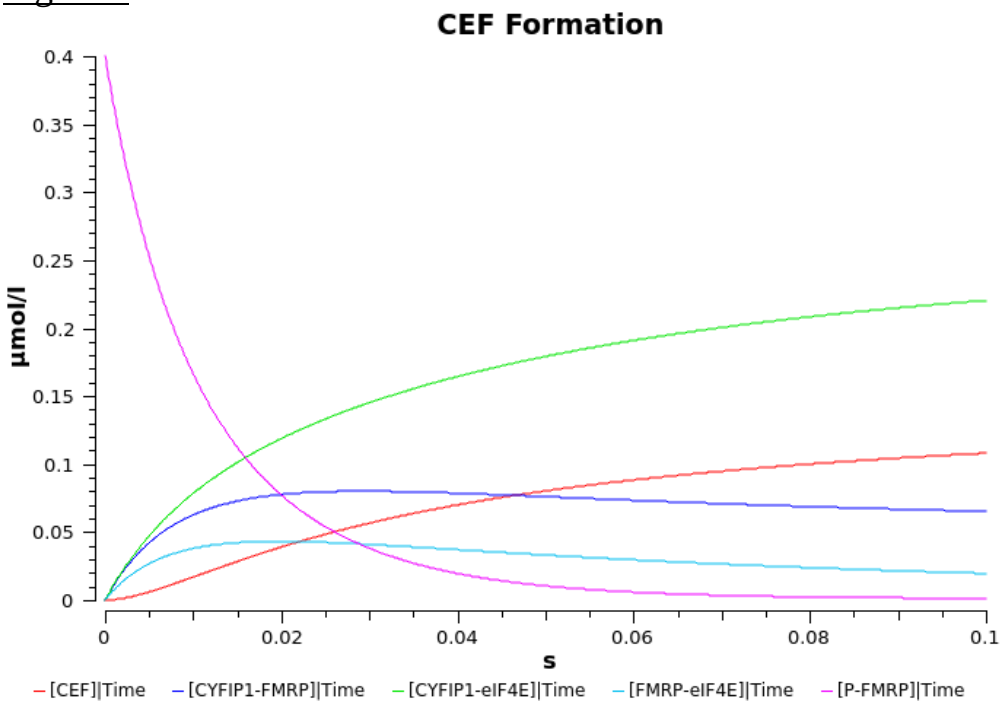
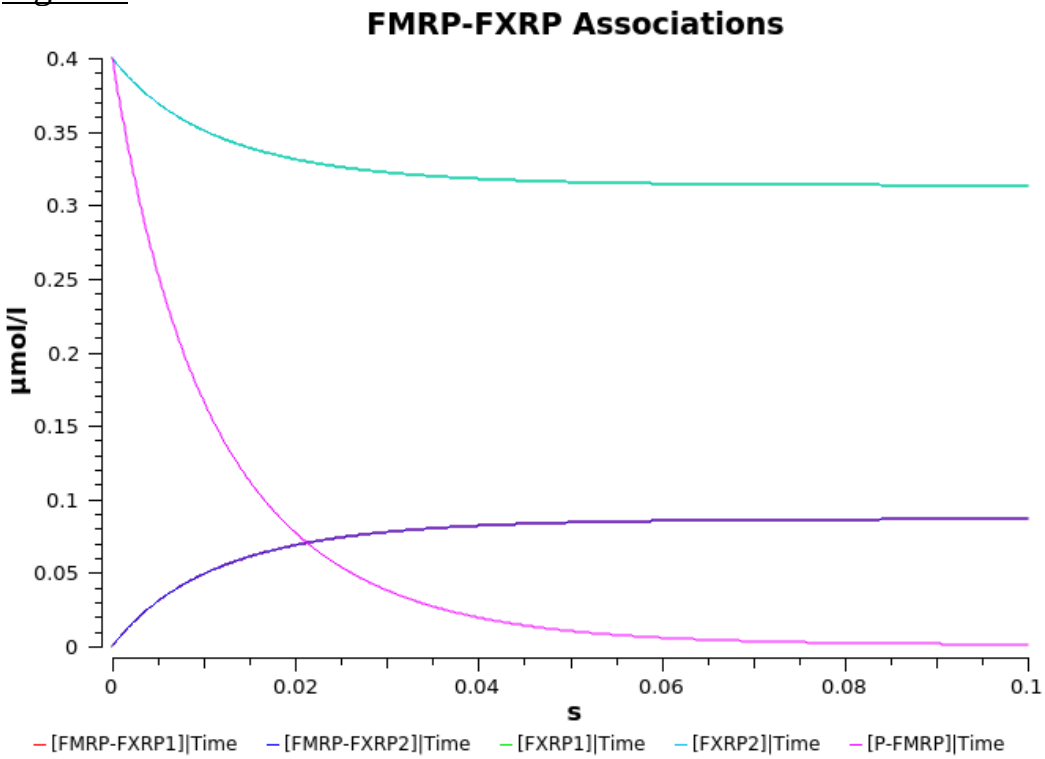


Figure 5



Note: FXRP1 and FXRP2 have the same concentrations and form the blue-green line; FMRP-FXRP1 and FMRP-FXRP2 have the same concentrations and form the purple line.

Figure 6

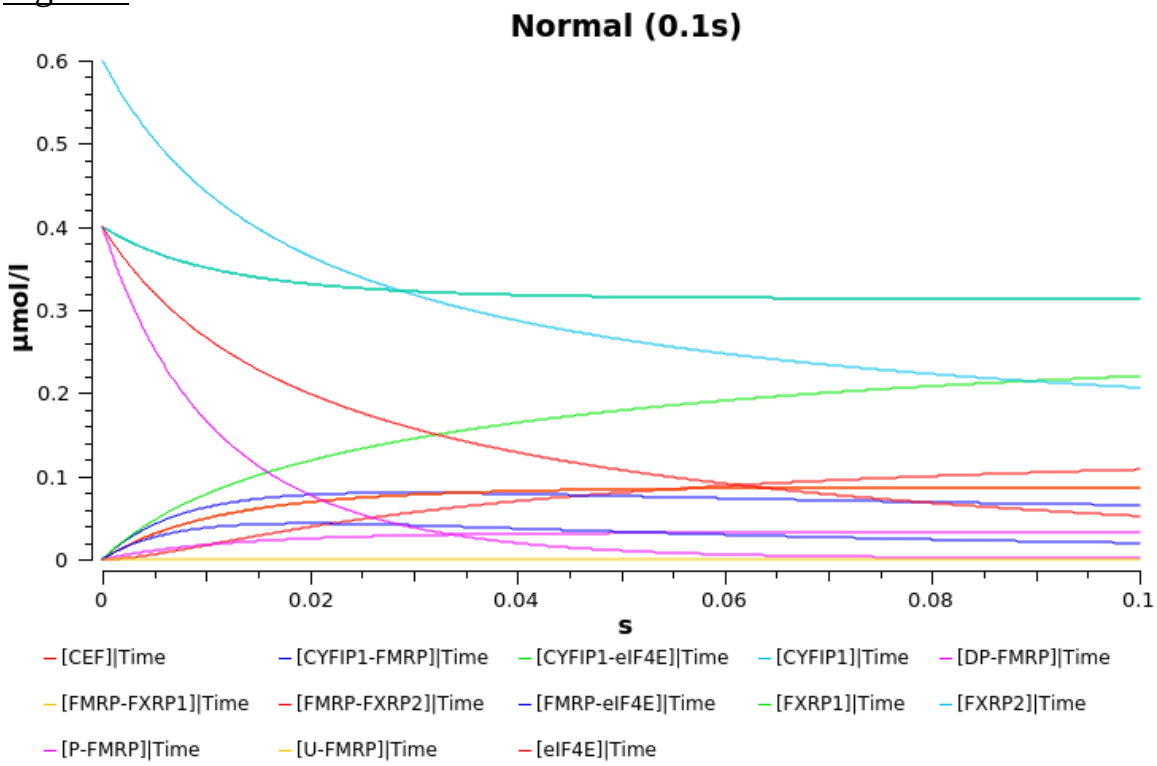


Figure 7

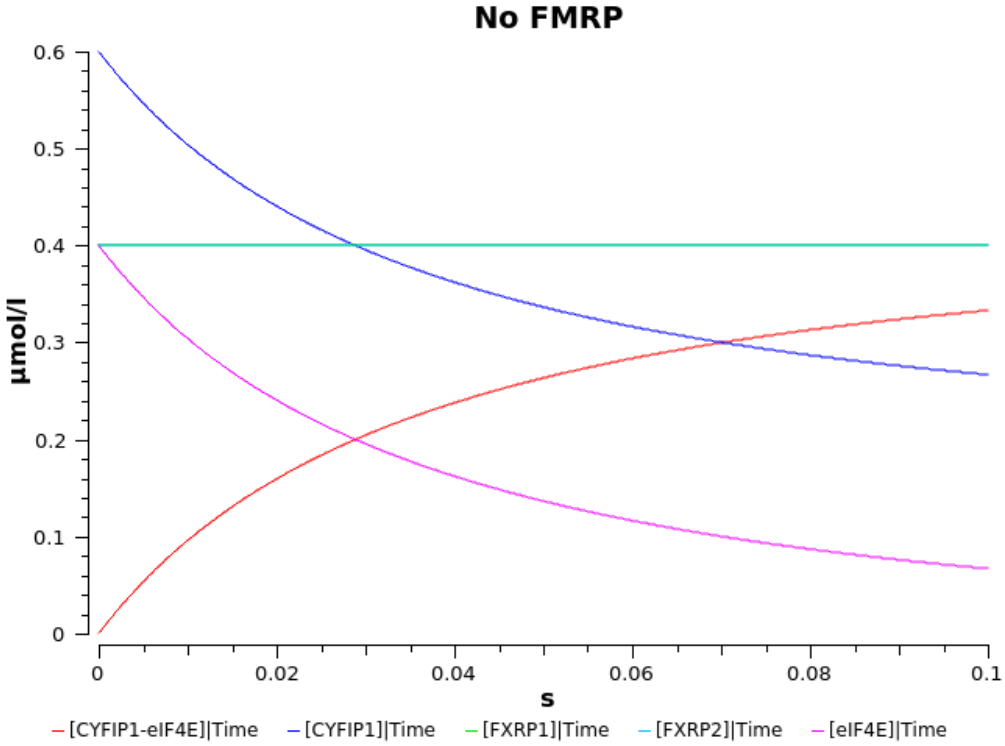


Figure 8

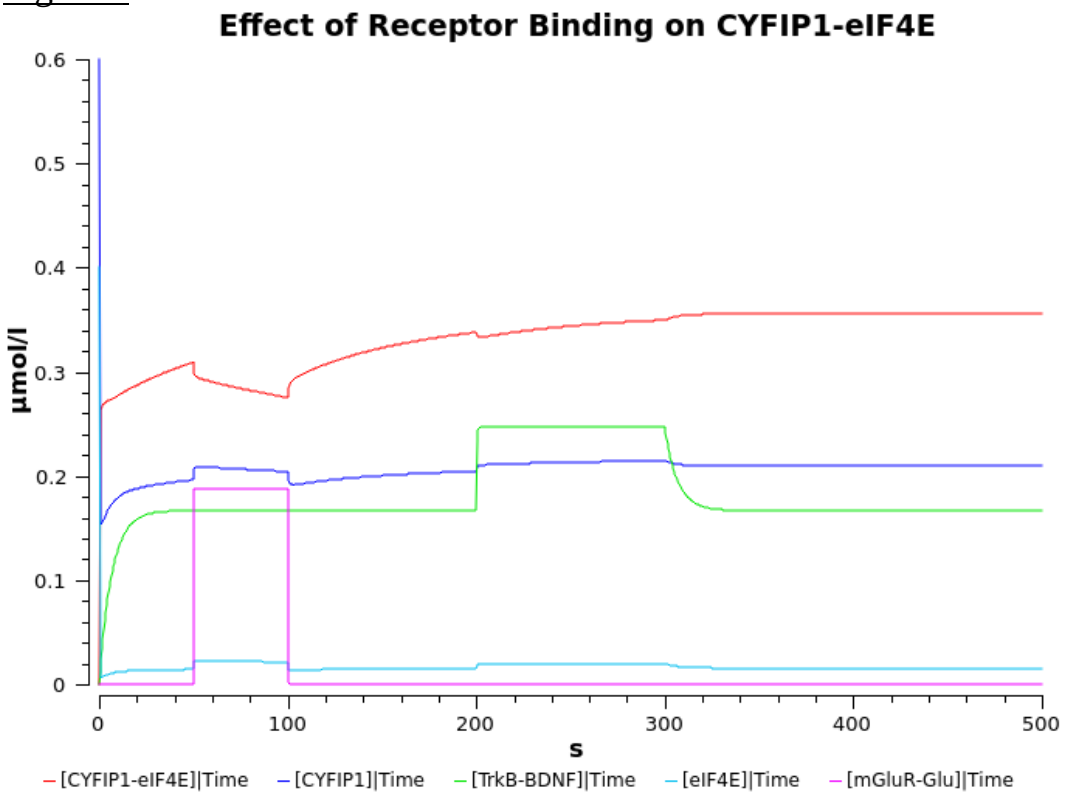
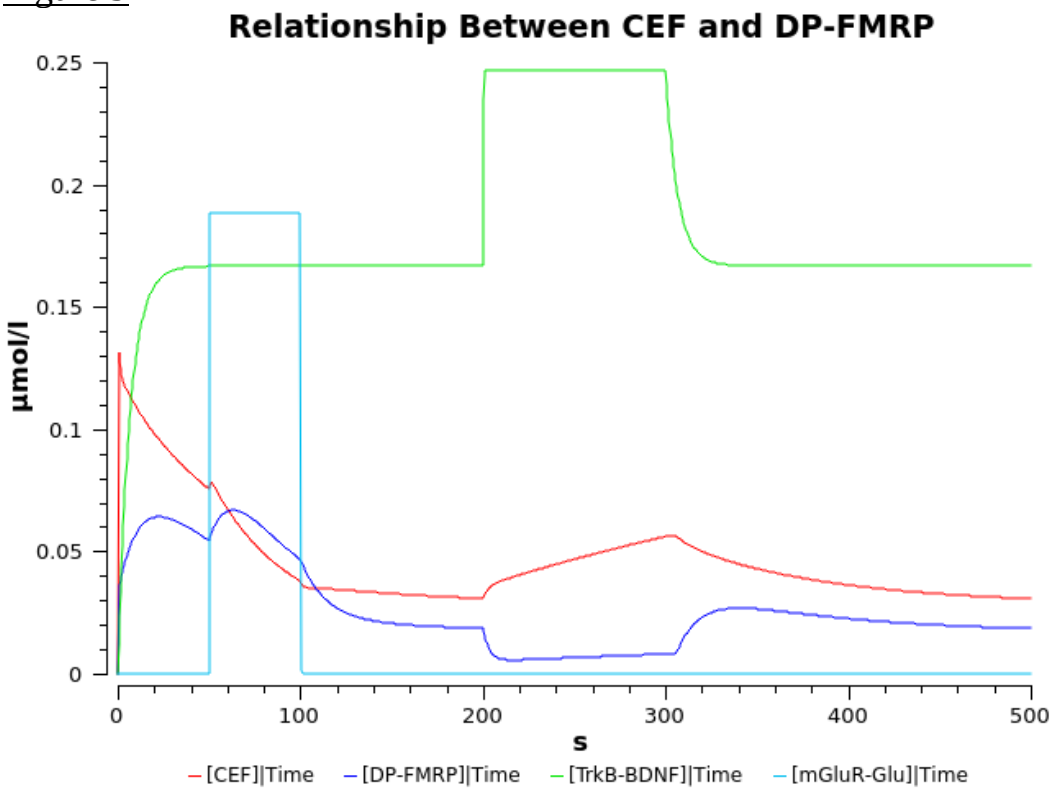


Figure 9



FMRP synthesis is the rate limiting step in the system

Figures 4 and 5 were created by running time courses of the model over 0.1s.

Figure 4 displays the concentrations of various CEF-related species over time and shows that CYFIP1-eIF4E reaches a higher concentration than CEF. This is significant as FMRP should bind to the CYFIP1-eIF4E to make CEF but FMRP quickly runs out of supply and so this does not happen.

Figure 5 displays the concentrations of FMRP and FXRP related species over time and shows that despite FXRP1 and FXRP2 being in high supply, the formation of FMRP-FXRP1 and FMRP-FXRP2 quickly stops as there is no FMRP to bind to FXRP1 or FXRP2.

Therefore it can be said that FMRP synthesis is the rate limiting step in several reactions in the system which confirms our hypothesis that FMRP is centrally important to the functioning of the system.

The functioning of the system is highly dependent on FMRP concentration

Figure 6 displays the concentrations of all non-constant species in the first 0.1s of the system. This can be said to be the normal operation of the system. In contrast, Figure 7 displays the concentrations of the same species but the parameters of the model are altered in such a way that FMRP is not present in the system and is not synthesised. This is reflective of the state of the biochemical system in the brain of FXS sufferers.

It can be seen that many species are not present in the system due to the loss of FMRP as it is either a component of these species or is a precursor to them. This suggests that FMRP is not only a translational regulator but a regulator of the system and this confirms our hypothesis that the regulation of FMRP leads to the regulation of the system as a whole.

Receptor binding events are key translational effectors

Figures 8 and 9 display Time Courses that were run for 500s so that we could investigate the effect of neurotransmitters binding to receptors on the post-synaptic neuron where all of our biochemical interactions take place. The first “rectangular” spike shows a sharp, temporary increase in mGluR-Glu receptor-neurotransmitter complexes and the second is for TrkB-BDNF complexes.

Figure 8 shows that mGluR-Glu binding causes a significant breakdown of CYFIP1-eIF4E (from ~0.32µmol/l to ~0.28µmol/l during the time period 50s to 100s) to its constituents. CYFIP1 shows a temporary increase from ~0.2µmol/l to ~0.22µmol/l and eIF4E shows a much smaller increase. This is also the case for TrkB-BDNF binding but it has a noticeably weaker impact as the CYFIP1-eIF4E decrease is small.

Figure 9 also displays the effects of receptor binding events but this time shows the effect on CEF and dephosphorylated FMRP concentrations. It can be seen that mGluR-Glu binding causes a drastic dephosphorylation (due to increased PP2A activity) of FMRP that is quickly rephosphorylated or ubiquitinated. Contrary to what we expected the event also causes a very small increase in CEF concentration compared to the decrease that we hypothesised would happen due to having less FMRP which is a constituent of CEF. It can also be seen that TrkB-BDNF binding causes a significant decrease in dephosphorylated FMRP (from ~0.02µmol/l to ~0.02µmol/l in the time period 50s to 100s) as S6K activity is increased and rephosphorylates the FMRP. This then causes a drastic increase in CEF (from ~0.03µmol/l to ~0.06µmol/l in the time period 200s to 300s) as the concentration of one of its constituents has increased. These results indicate that our hypothesis that receptor binding events have a large impact on the system is true and that this impact is the regulation of CEF concentration.

Discussion

By the end of my Nuffield Research Placement I have managed to successfully develop a computational model of FMRP regulation. The model is much larger and more detailed than we had anticipated at the beginning of the placement and due to the presence of receptors and certain enzymes it can be easily integrated into larger models.

The main limitation on the accuracy of the model is the lack of experimental data on FMRP and related compounds. Many of the concentrations and kinetic values in the model are estimates based on similar compounds and reactions that we currently do have data for, and so having the actual values would make the model much more reliable.

That being said, we have obtained some quite significant results from running the model with various parameters. Our finding that FMRP synthesis is the rate-limiting step in the system confirms the importance of FMRP as it has so many binding partners. Our second result that the functioning of the system is dependent on FMRP concentration also confirms the importance of FMRP in the system but also suggests that Fragile X Syndrome is not only caused by the lack of FMRP being a translational repressor, but that the lack of FMRP has an adverse effect on other molecules and reactions in the system. Our third result that receptor binding events are key translational effectors, is maybe our most significant result. The increase in CEF concentration due to TrkB-BDNF binding may suggest that in conditions where there is still FMRP but very little of it, the activating of the TrkB receptor (either by BDNF or an agonist) may cause an increase in CEF concentration and thereby increased

translational repression. This could be quite dangerous as the TrkB receptor has many other functions than regulating CEF (Fariñas et al., 1998) but it is still a potential avenue to follow. Due to the nature of computational models, there are still many more hypotheses that can be tested on the model in the future such as further investigation into the relationships between FMRP, receptor binding events, and enzymes; and possibly looking into changing rates of ubiquitination or degradation to investigate the viability of possible treatments for FXS.

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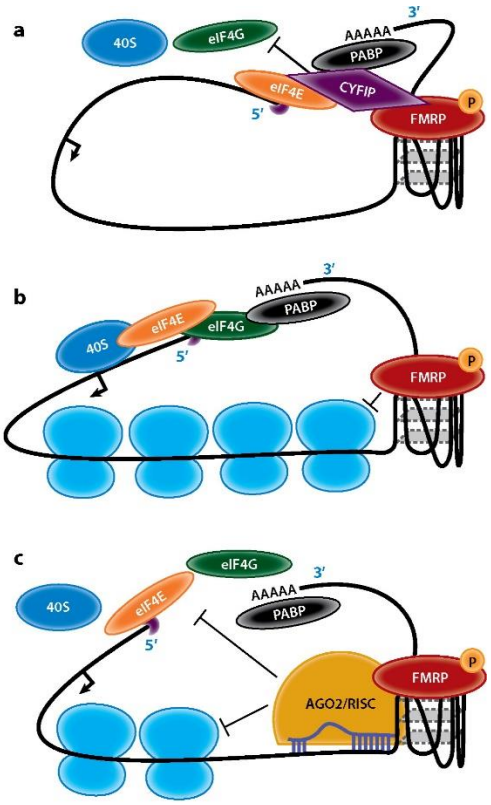
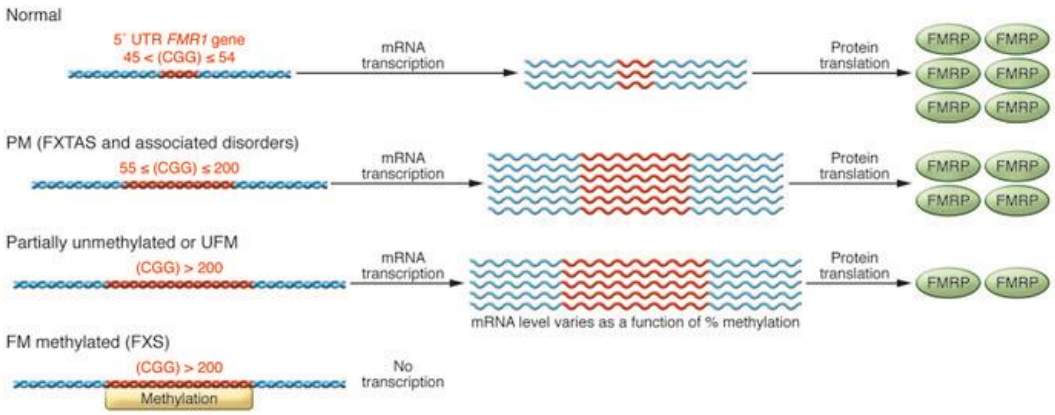
Appendix 1



Santoro MR, et al. 2012.
Annu. Rev. Pathol. Mech. Dis. 7:219–45

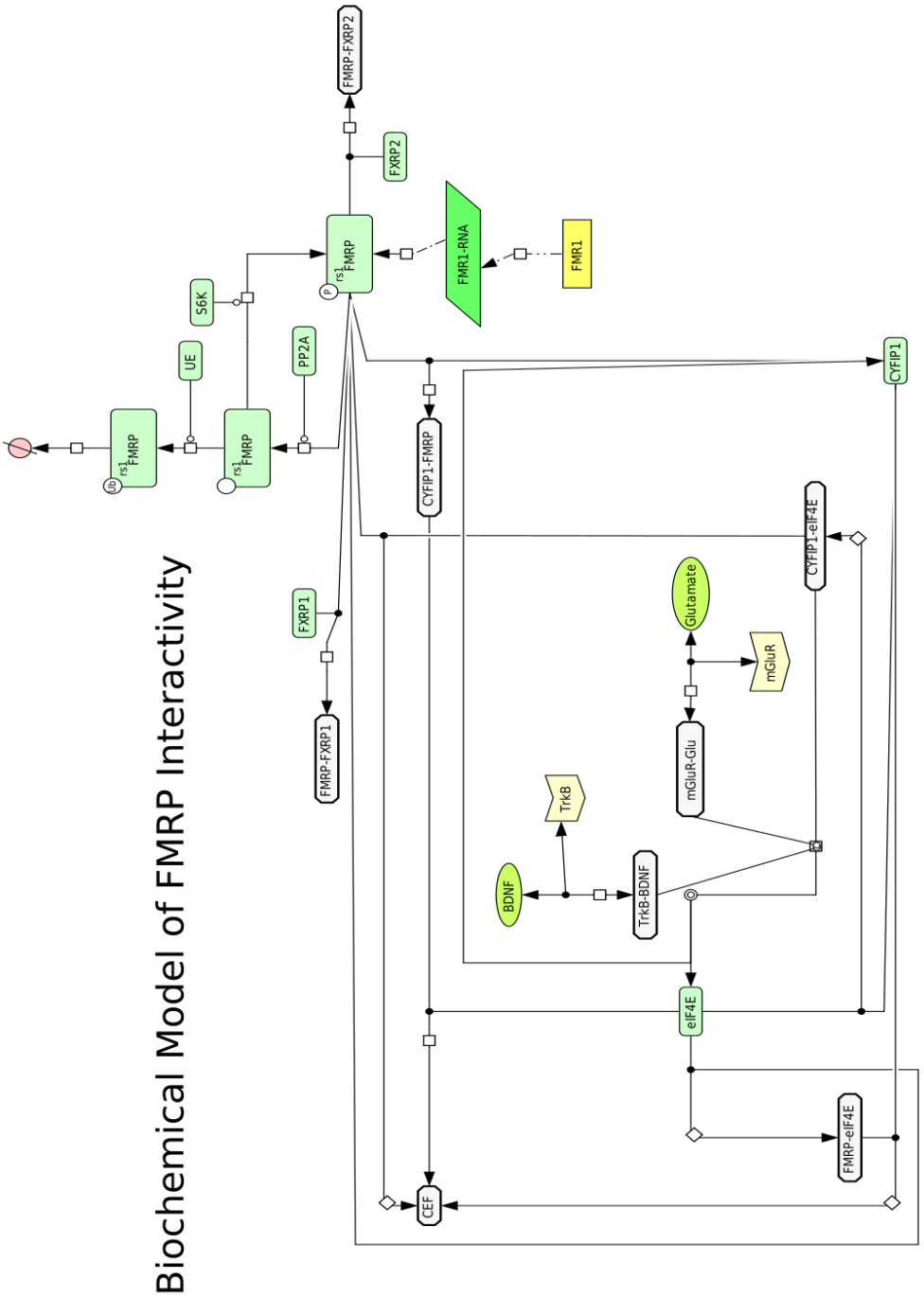
FMRP acts as a translational repressor as a member of the CEF complex. Adapted from Santoro, Bray and Warren, 2012.

Appendix 2



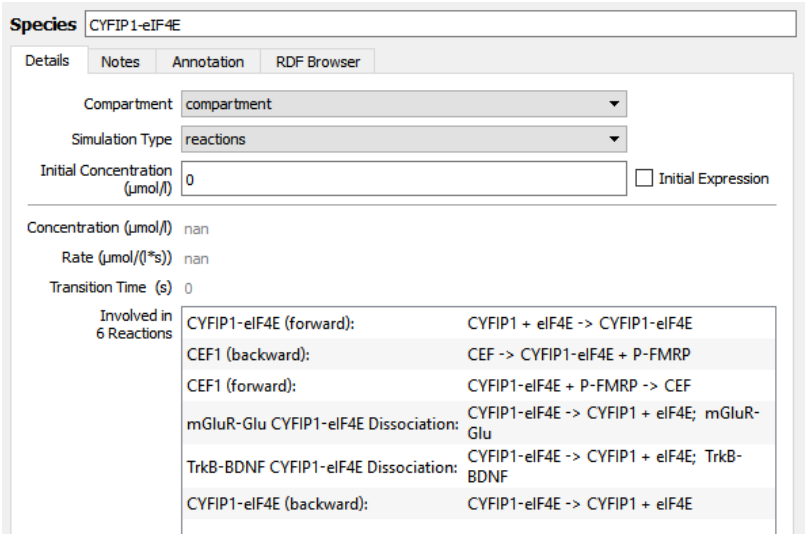
Various levels of CGG repeat and their effects. Adapted from Bagni et al., 2012.

Appendix 3

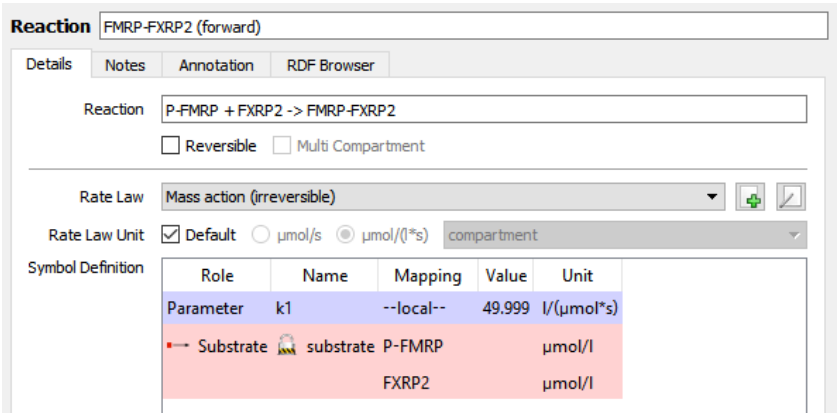


A visual representation of the reactions in my computational model.

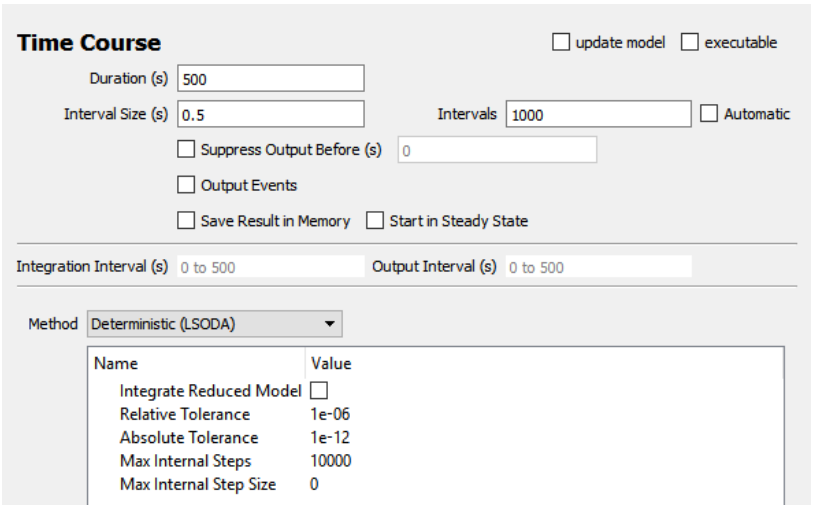
Appendix 4



The COPASI UI for species



The COPASI UI for reactions



The COPASI UI for Time Courses