Modelling Nitric Oxide signalling pathways in neuron

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Abstract

Many modelling methods have been developed to simulate the nature of chemical signalling pathways that occur in the brain at the neuronal level. For this project, I used the tools developed in the lab, mainly HillTau, FindSim, HOSS and MOOSE to create an abstracted as well as a detailed enzymatic mass-action model to demonstrate the reaction pathways for the production of Nitric Oxide (NO) and its role in downstream activation and regulation of multiple molecules, proteins and gene expression necessary for the normal functioning and activity of a healthy neuron. The parameteric values of the model are based on the experimental data validation with an attempt to produce simulation results as close to the experimental data as possible. These models can be used to simulate how different molecular stimulis affect the production of other molecules in the cascade present in the model with variable time and concentrations.

1 Introduction

The junction formed between the two neurons, called the synaptic cleft, signals the channelling of multiple chemicals as neurotransmitters from the pre-synaptic neuron to the post-synaptic neuron [KSJ91]. These chemicals are involved in the various downstream chemical signalling in the neurons, which play a significant role in multiple neuronal processes and activities [KSJ91]. Many experimental studies surrounding the hippocampal neurons have shown the role of molecules like dopamine [AHP07], nitric oxide [HDF13], serotonin [Juh98] etc., in regulating and modulating major biological functions like neurogenesis [HDF13], apoptosis [iOL15], etc. Disturbances in these signalling pathways have shown extreme consequences leading to neurodegenerative and psychological diseases like Parkinson's disease[KSJ91], Autism[KSJ91], Alzheimer's [KSJ91] disease etc. Insights into these pathways and information about production of multiple molecule signalling can help us understand the defects and differences in a healthy and a diseased brain. These insights can guide our knowledge to study how we can design specific drugs to modulate the concentration of different molecules during the over or under-production of essential chemical molecules in a diseased condition.

One such molecule essential in the hippocampal neuron signalling and involved with the regulation of multiple pathways is Nitric Oxide (NO), which is a gaseous neurotransmitter and messenger and is known to regulate hippocampal neuron neurogenesis [HDF13],[KSJ91], cell death by apoptosis [iOL15],[Juh98] and DNA damage and repair [PPGBM19]. Experimental results have successfully shown the role of NO in activating molecules like Guanosine 3',5'-cyclic monophosphate (cGMP) and phosphorylated cGMP response element-binding protein (pCREB) for neurogenesis [HDF13],[KSJ91]. S-nitrosylation by NO of enzymes like Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is responsible for many housekeeping functions and cell death [iOL15],[Juh98]. Radical reactions in the presence of oxygen convert NO to peroxynitrite form ($ONOO^-$), which initiate multiple responses during DNA damage and repair mechanisms [PPGBM19].

These biochemical signalling pathways are highly dynamic in nature. It is difficult to experimentally determine how the concentration of each molecule is dependent on other molecules and how their productions are interdependent and co-related in-vivo. Hence we use modelling methods to simulate the mechanisms to study and better understand how these signalling pathways relate to the production of different molecules and develop with time, and how they are affected in the presence of molecular drugs. Two methodologies to go about modelling these pathways were explored throughout the course of the project. First was modelling the pathways by abstracting the role of the molecules to display a simplistic representation of the interactions depending on fewer parameters using HillTau [Bha21b]. Later a more detailed model was created, which involved the mass-action kinetics in segregating the

role of enzymes and other binding mechanisms involved in the reactions with detailed parameters using MOOSE [Bha15]. These models are based on the literature curation of experimental data, and the parameters were determined based on the experimental results by fitting in those data with the simulation presented by the models using FindSim [VHSB18]. Since experiments provide in-vitro results, their co-dependence and validity vary amongst different data, and hence the parameters need to be optimised according to the most optimal fits with scores as close as possible to the real-life data, which was done using HOSS optimiser [Bha21a]. These models provide the simulation of the production of different molecules involved in the whole signalling pathway in response to different stimuli and their evolution with time, based on experimental data and validation.

2 Methodology

2.1 Abstraction of Model

The HillTau model is based on the abstraction of signalling pathways by involving fewer parameters using minimalistic details and constraints to reproduce a biologically relevant network output in-silico. It uses the Hill equation, the Michaelis-Menten enzyme-substrate kinetics, and the tau equations to describe the parameters involved in the mechanism of the reaction and saves time from calculating the ordinary differential equation for each molecule. To create the HillTau model for the representation of the signalling pathways regulated by NO, a detailed literature review [HDF13],[KSF21],[HZ14],[Ben11], [iOL15],[PPGBM19],[TSWG11] was carried out to determine the molecule's production and its role in the regulation and down-streaming process of the further molecules.

To write the script for the HillTau, the parameters considered for each molecule are the association constant (K_A) (Eq. 1), tau value for forward reaction (τ) (Eq. 2), tau value for backward reaction (τ_2) (Eq. 3), modifier kinetics (Eq. 4), the initial concentration of the substrate molecules and their role as an inhibitor, if any. The connections throughout the models are based on the substrate and enzyme provided in the script, which varies according to the number of molecules, the type of interaction they exhibit (inhibitory or stimulatory), or their action as a modulatory molecule [Bha21b].

$$Y_\infty = Y_{\text{input}} \frac{L^n}{KA^n + L^n} \tag{Eq. 1}$$

$$\text{If } Y_\infty > Y(t) \text{ then } \frac{Y(t+\Delta t) - Y(t)}{Y_\infty - Y(t)} = 1 - \exp\left(\frac{-\Delta t}{\tau}\right) \tag{Eq. 2}$$

$$\text{If } Y_\infty < Y(t) \text{ then } \frac{Y(t+\Delta t) - Y(t)}{Y_\infty - Y(t)} = 1 - \exp\left(\frac{-\Delta t}{\tau_2}\right) \tag{Eq. 3}$$

$$Y_\infty^{\text{mod}} = \frac{Y_{\text{input}} L^n}{L^n + KA^n \frac{(1+\frac{M}{K_{\text{mod}}}{}^h)}{(1+A_{\text{mod}}\frac{M}{K_{\text{mod}}}{}^h)}} \tag{Eq. 4}$$

2.2 Experimental Curation

The parameter values inputted in the model are determined based on experimental data and validation. FindSim curates the experimental results and integrates the HillTau model to simulate the experiment based on the parameters. A map file can match the molecules from the model file to readout in the experimental file and combine it together for simulation of the experiment. A large database of experiments helps with better results in determining the parameters, so that when optimised, they can

be considered more optimally correct to the actual values. The output shows the graph of experimental and the simulated results for specific stimuli and assigns a score to define the difference between the two plots based on Normalized Root Mean Square (NRMS) values [VHSB18].

Finding experiments involve extensive literature review and database analysis. The valuable data in the experiments are based on the different stimuli to check for the readout of signalling molecules in a dose-response or time-series-dependent manner. This collection of experiments for the model needs to be such that it covers the stimuli and readout molecule at close proximity to each other as well as depicts a further away relation for better optimisation.

2.3 Parameter Optimisation

The parameters in the model are determined such that they belong to the biological range and align with the experimental data collected for the molecules involved in the model. HOSS optimisation [Bha21a] is used to optimise these values, which uses the Constraint Optimization By Linear Approximation (COBYLA) algorithm [Pow94] to produce optimised parameters for the model based on the experimental database. The config. file is described to consider the overall weights of different experiments based on experimental details and characteristics like the reader's assessment, organism, cell type, temperature, and time observed as suited to the modeller's perspective (Fig. 6) and optimises it accordingly. These optimisations are done in a group-wise manner to optimise the groups downstream in the model in accordance with the upstream optimised values. Multiple rounds of optimisation are carried out along with parameter bounds to find suited values with the least scores for individual groups and the overall model.

Once a model with an optimised parameter value has been created, it can be run on the HillTau script to see the concentrations of molecules produced with time based on different substrates and stimuli. This in-silico model to study the production of molecules can be used to determine how the concentration of one molecule affects the whole cascade and how the presence of additional molecules can affect the whole network.

2.4 Mass-Action Model

The HillTau model is a simple abstraction of a detailed cascade and can be used to create a comprehensive model consisting of mass-action enzyme kinetics (Reac. 1) and reaction binding-unbinding rates. This type of model was created using the MOOSE script [Bha15] to define the connection of the molecules in the model and define parameters based on intrinsic details like the rate constant of forward reaction (K_f), rate constant of backward reaction(K_b), Michaelis-Menten constant (K_m) (Eq 5), and the catalytic rate constant of the substrate to product (K_{cat}) (Eq 6). These are converted to the GENESIS or SBML format to be interpreted for further visual representation and mapped to the FindSim experiment simulations. Since the molecules and kinetics involved in this model are more detailed than the abstract model, more detailed experiments were collected for better scores and results.

$$[\text{E}] + [\text{S}] \rightleftharpoons [\text{ES}] \longrightarrow [\text{E}] + [\text{P}] \tag{Reac. 1}$$

$$\nu_0 = \frac{V_{\text{max}} S}{K_m + S} \tag{Eq. 5}$$

$$V_{\text{max}} = [E] K_{\text{cat}} \tag{Eq. 6}$$

The mass-action model is further analysed and optimised in the same manner as the HillTau model by HOSS optimisation. This was done initially by trying to optimise K_f, K_b, K_m and K_{cat} and later with K_d, tau, K_m and K_{cat}. The experiment and simulated plot were plotted to determine the optimal parameters for the model.

3 Results

3.1 HillTau Model

The HillTau model for the production of Nitric Oxide (NO) and its role in regulating different signalling pathways was created (Fig. 1). The parameters were optimised according to the collected experiments (26 experiments)(Fig. 7) to find the best overall score of the model after optimisation such that the production of molecules was within the biological range for different substrates and products (Fig. 2). The final score for the parameters of the whole model was 0.2486 which suggests that the simulation of the experiment fits were good enough to be considered for the optimal model values in accordance to the experimental data. The scores of the individual groups involved in the whole model are also shown (Table 1).

Group Name	Score
Calmodulin Kinase	0.1296
Nitric Oxide	0.4488
HC Neurogenesis	0.3348
Apoptosis	0.1481
DNA Repair	0.1819

Table 1: Final scores of the experiment compilation after optimisation per group for the NOS HillTau model

3.2 FindSim Experiments for the HillTau model

After selecting the values of the final parameters for the model given by the final optimisation results along with manual tweaking, The results of the FindSim files were plotted to compare how the simulation results differed from the experiments (Fig. 3).

3.3 Mass-Action Model

The mass-action model is the detailed version of the HillTau model involving the binding reaction (shown by squares) and enzymatic reactions (shown by ellipses) (Fig. 4). Some of the parameters were initialised and taken from the existing DOQCS database [HRB13] while the rest of the parameters were optimised according to the collected experiments (50 experiments) (Fig. 7) to find the best overall score of the model after optimisation such that the production of molecules was within the biological range (The concentration-dependent production of molecules with time can be analysed using MOOSE-GUI). The final score for the parameters of the whole model was 0.3898 which suggests that the simulation of the experiment fits were fine enough to be considered for the optimal model values but can be made better by optimising it with more experimental data. The scores of the individual groups involved in the whole model are also shown (Table 2).

Group Name	Score
Ca	0.1930
CaM	0.4486
CaMKII	0.2936
PP1	DOQCS
NO	0.3886
HC	0.4398
Ap	0.4332
DR	0.5321

Table 2: Final scores of the experiment compilation after optimisation per group for the NOS mass-action model

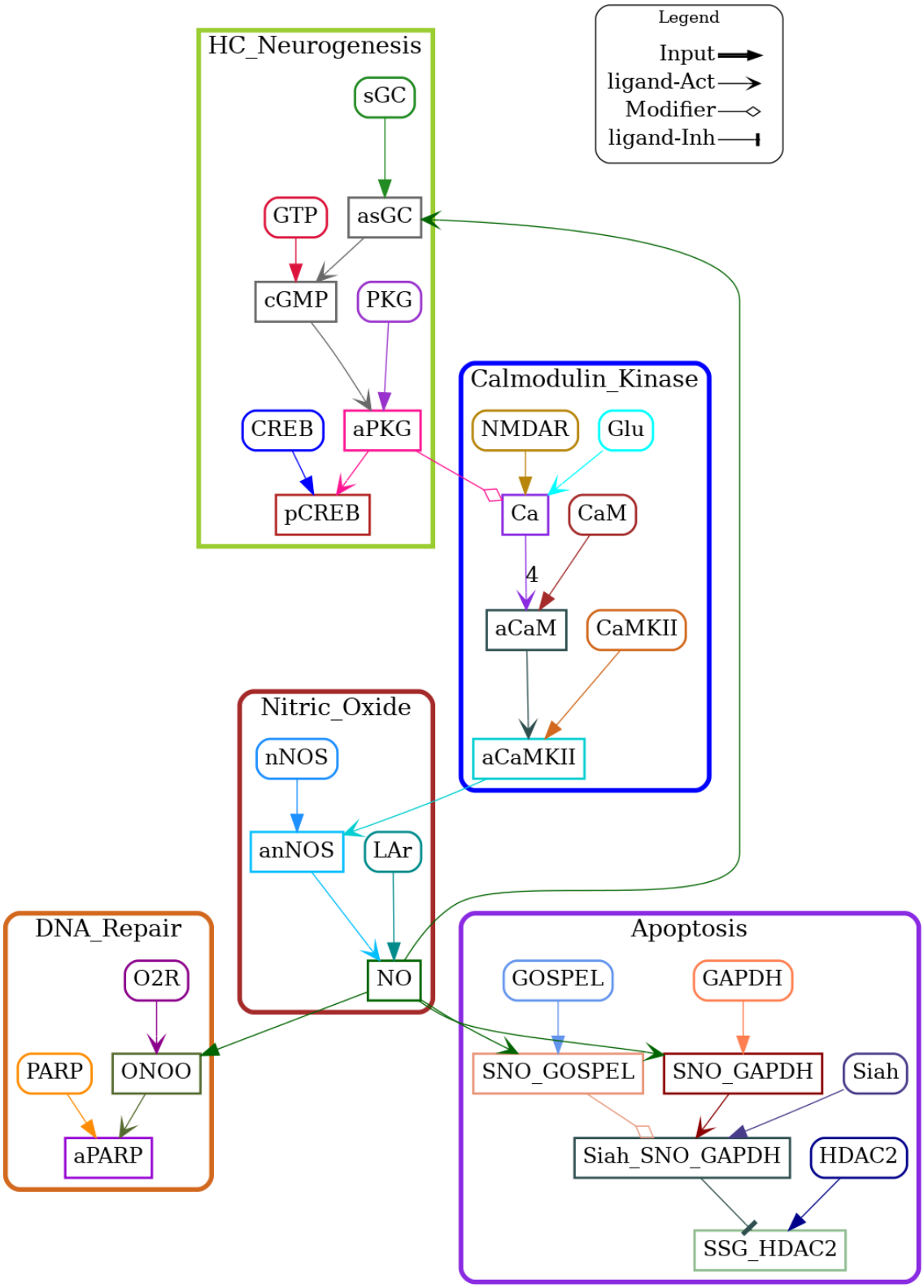


Figure 1: NO signalling model using HillTau

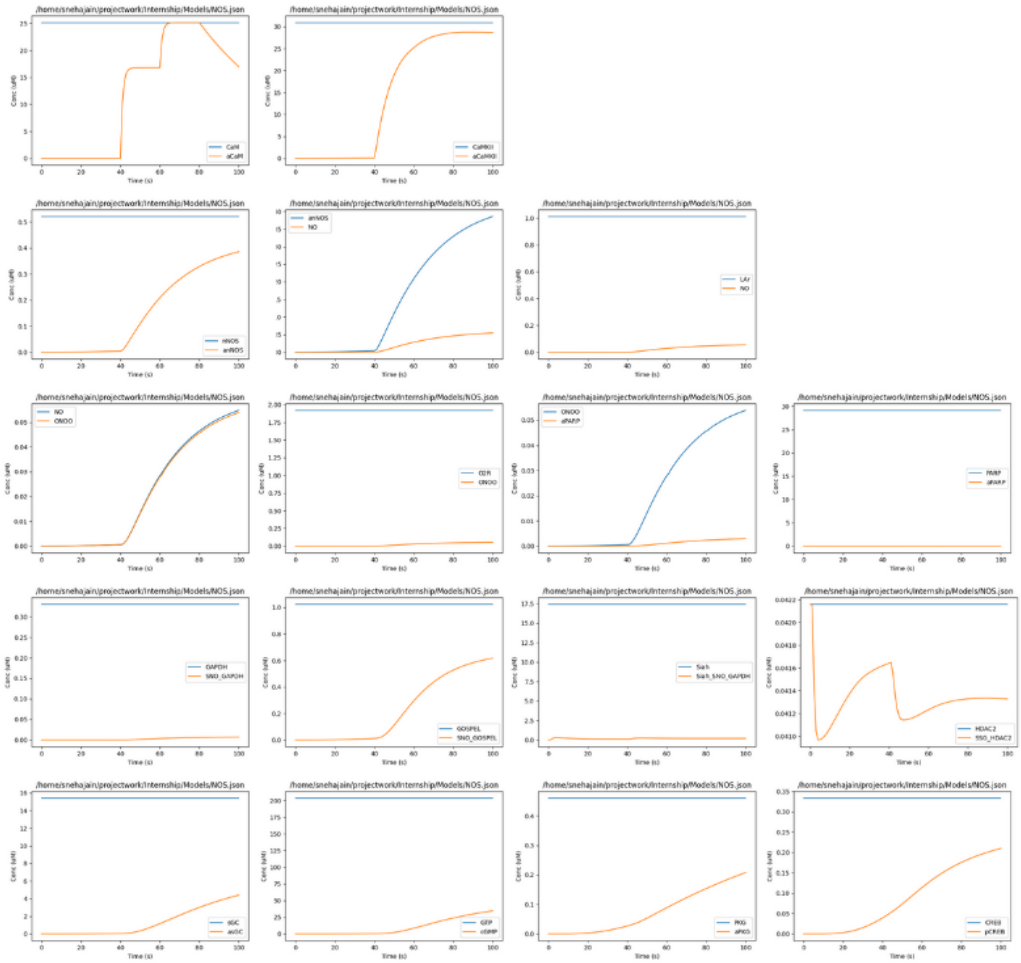
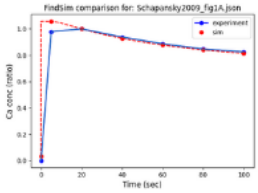
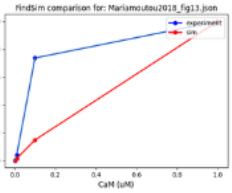
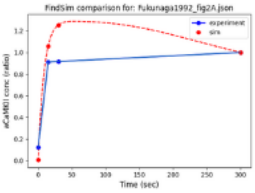


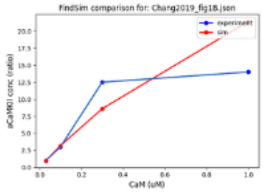
Figure 2: HillTau model results for substrate-product concentrations with time for different molecule

Calmodulin Kinase

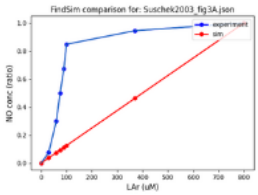


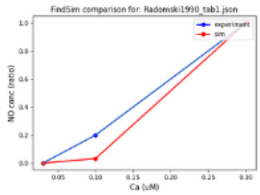


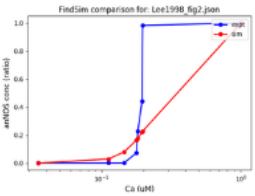


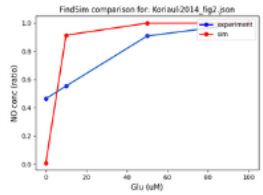


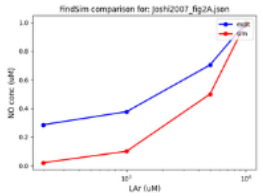
Nitric Oxide

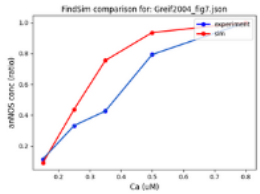




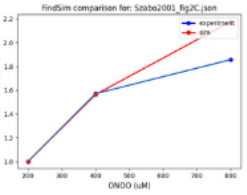


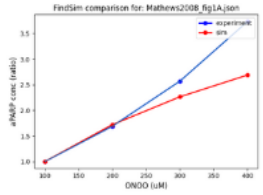




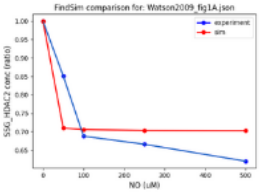


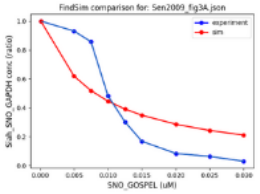
DNA Repair



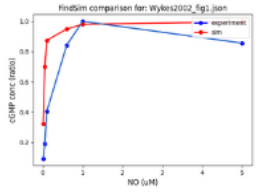


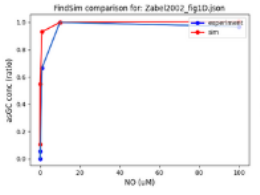
Apoptosis

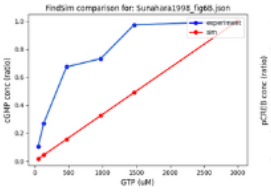


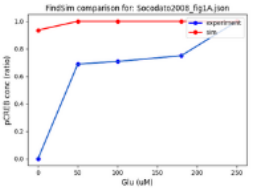


HC Neurogenesis









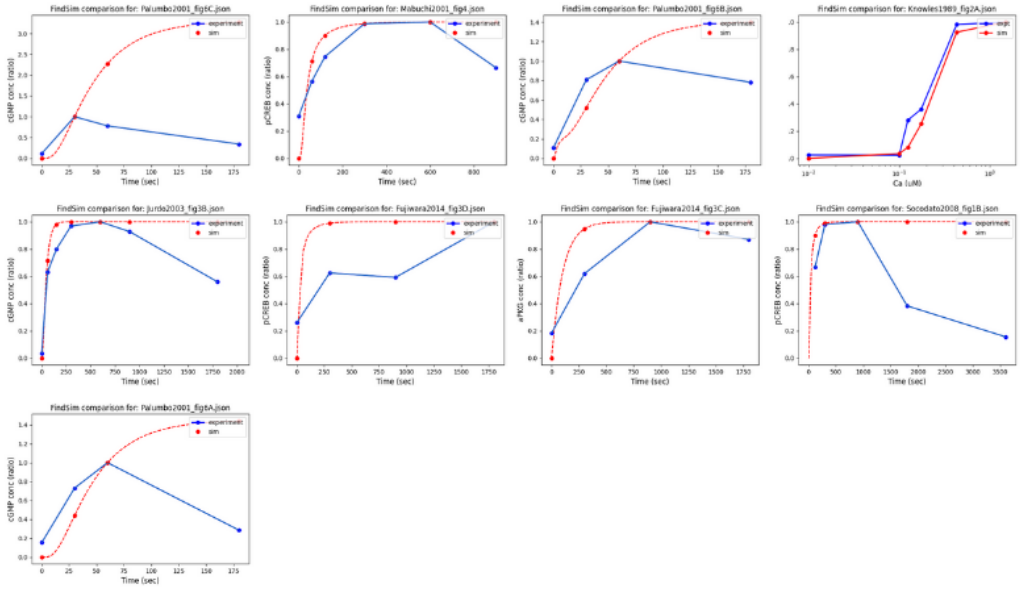


Figure 3: FindSim files showing experimental and simulation results after parameter optimisation

3.4 FindSim Experiments for the Mass-Action Model

After selecting the values of the final parameters for the model given after the optimisation and from the DOQCS database, The results of the FindSim files were plotted to compare how the simulation results differed from the experiments (Fig. 5).

4 Discussion

The brain is a centre of multiple signalling cascades regulated by neurotransmitters and messengers actions, and it is experimentally challenging to study how they are affected by other molecules involved in the pathways as well as the impact of the addition of external molecules in modulation of these reactions. All these caveats suggest a need to use computational and modelling methods to examine and develop a better understanding of these processes for clinical and psychiatric implications.

Here in this project, I tried to model the multiple signalling pathways regulated by the production of a gaseous neurotransmitter, Nitric Oxide (NO). NO is known to be an essential molecule in the activation of multiple downstream protein signalling [HDF13],[KSJ91]. It can cause S-nitrosylation of certain enzymes to initiate regulatory mechanisms [iOL15],[Juh98]. It also undergoes peroxynitrite formation under stressful conditions to regulate gene expression for cell survival [PPGBM19]. Modelling the production of proteins involved in these mechanisms gives us insights into how they are affected in a diseased neuron as compared to a healthy neuron and can be used to study how the concentration of one molecule affects the whole signalling cascade during various diseases. These can further be used to add to the implication of drug molecules on how they can regulate the pathways in a diseased neuron and provide major insights into the drug action.

I first developed an abstracted (D3) model to show the production and the role of NO in different processes using the HillTau model [Bha21b]. The decision and choice of parameters for the model to describe the various production of molecules are based on experimental validation. This is done by comparing the experimental and the simulated data results for different dose-dependent and time-series experiments known to exist using FindSim [VHSB18] and optimised further to fit the scores and weights of all the experimental databases as closely as possible using HOSS [Bha21a]. Further, a detailed model to describe the enzymatic and reaction mechanisms of a mass-action model (D4) involving in-

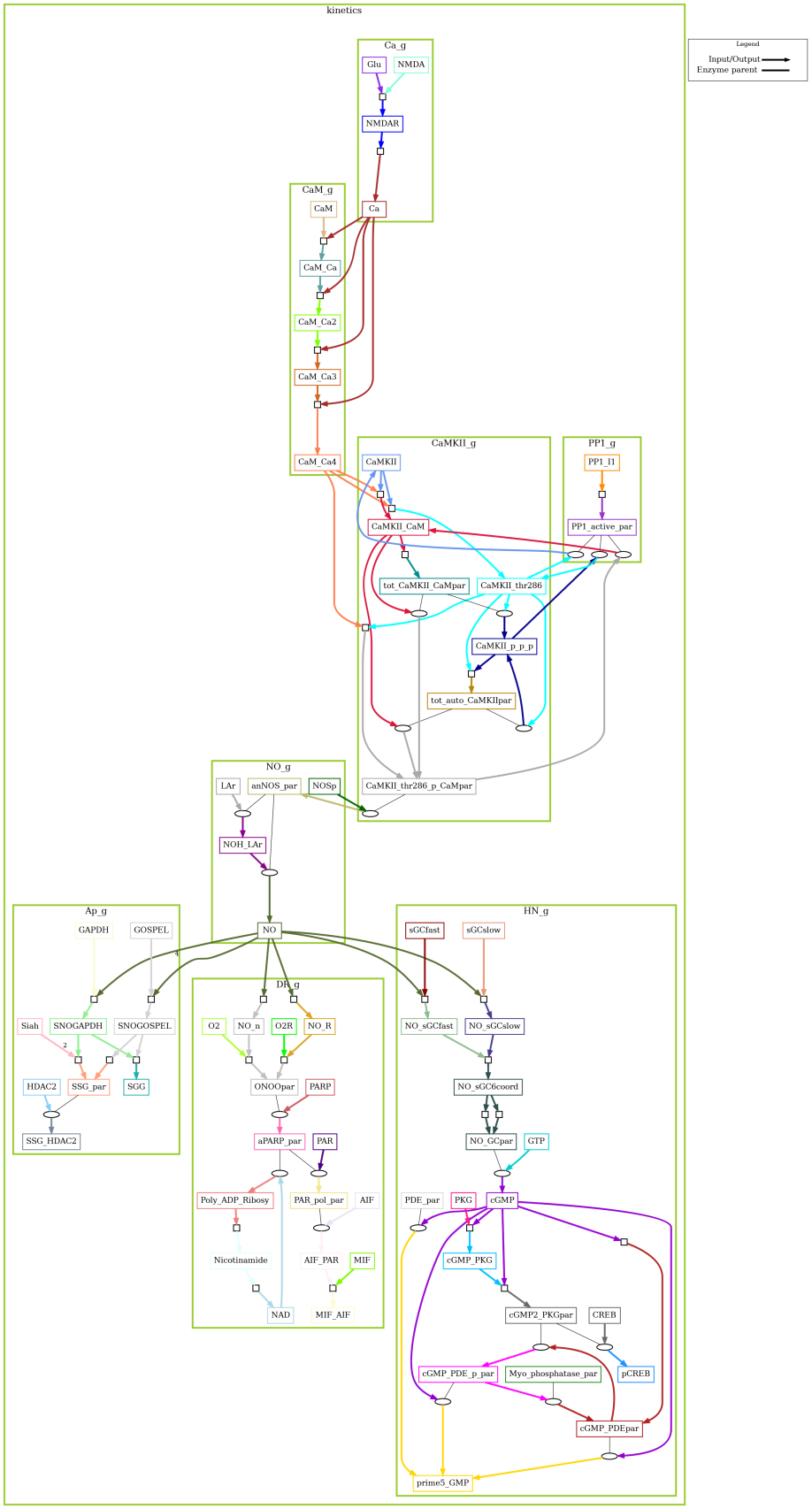
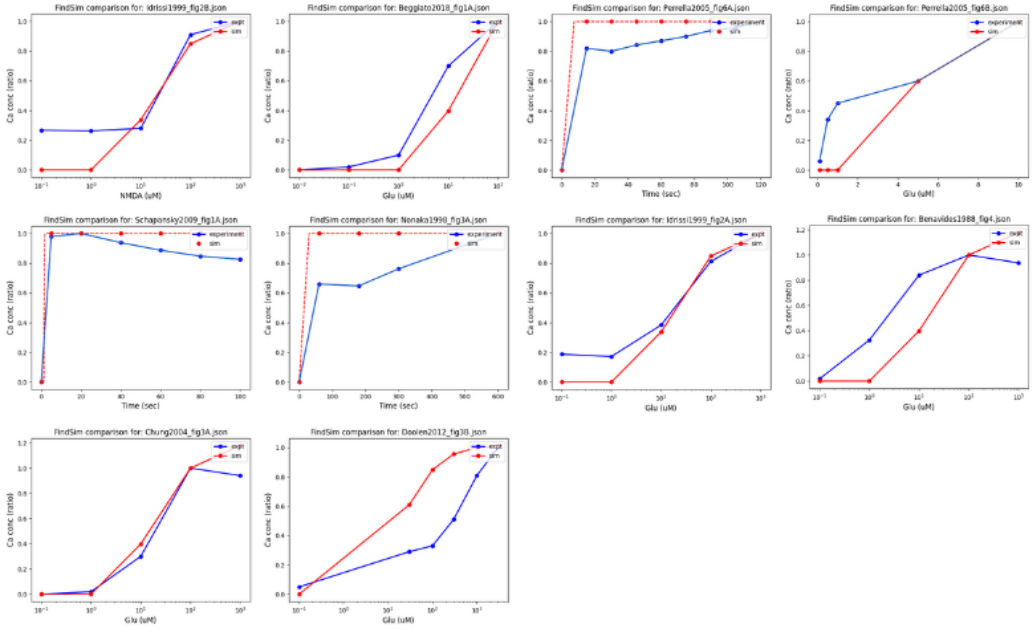
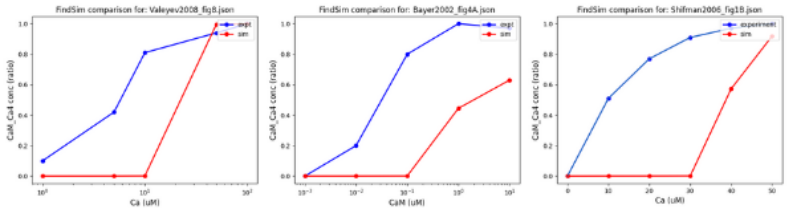


Figure 4: NO signalling model using MOOSE

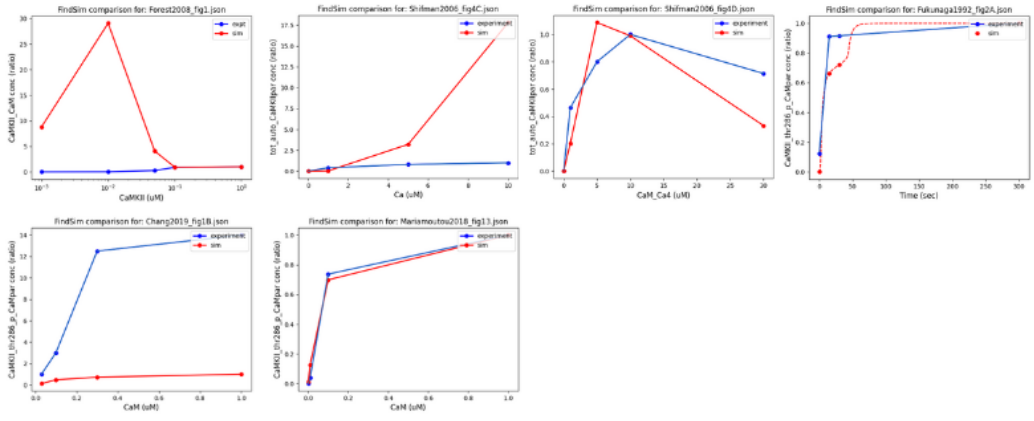
Ca_g



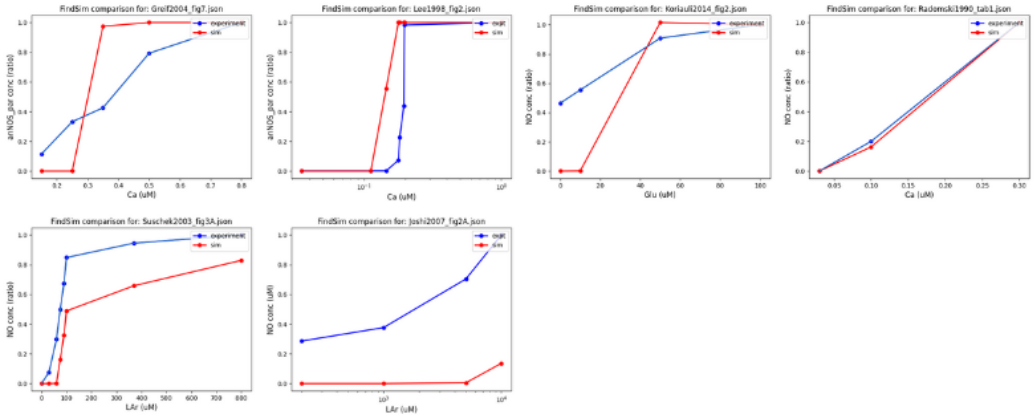
CaM_g



CaMKII_g



NO_g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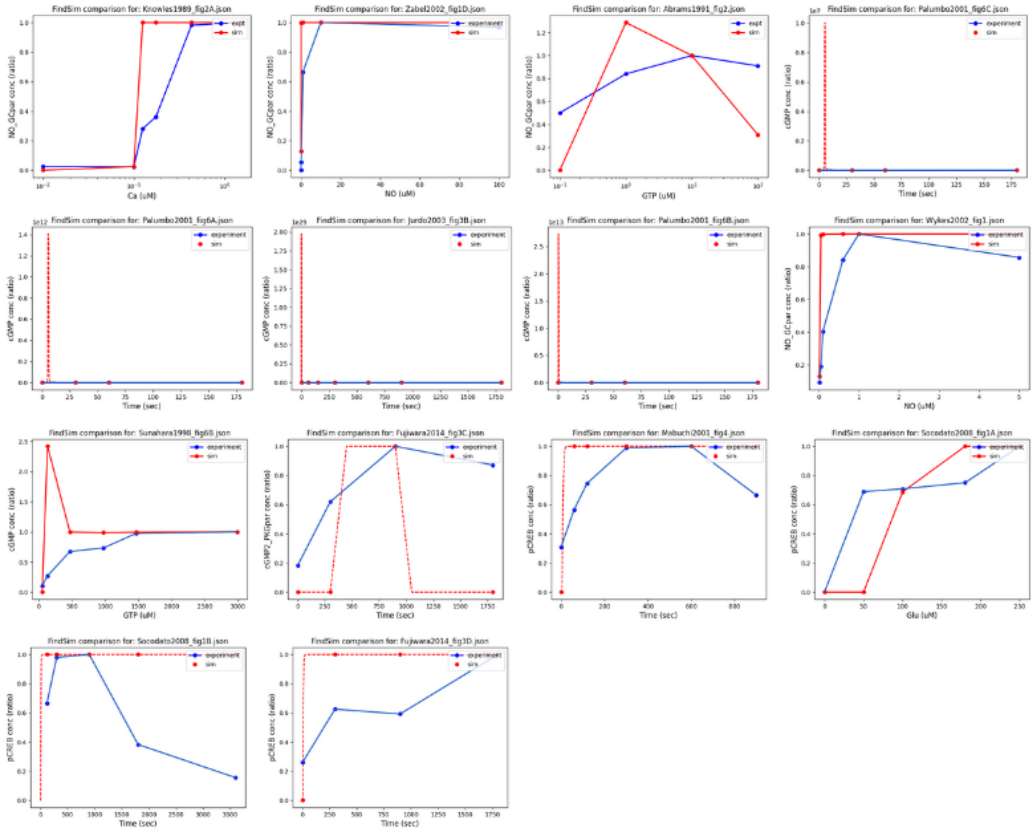
experiment
sim
Ca (uM)
Glu (uM)
LAr (uM)
NO conc (ratio)
NO conc (uM)

HN_g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experiment
sim
Ca (uM)
NO (uM)
GTP (uM)
Time (sec)
Glu (uM)
cGMP conc (ratio)
pCREB conc (ratio)

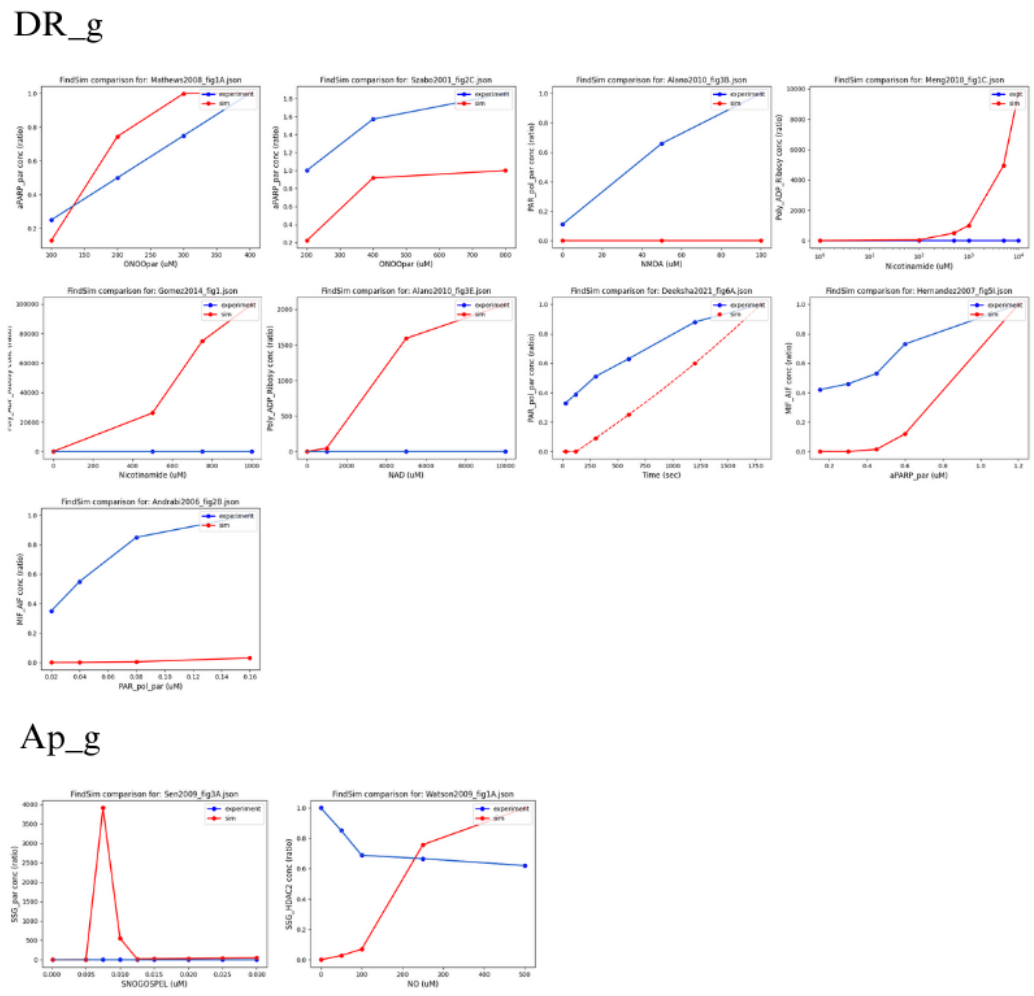

Figure 5: FindSim files showing experimental and simulation results after parameter optimisation

depth molecular binding and complex productions in the whole signalling pathway was created using MOOSE [Bha15]. These models represent a more realistic interpretation towards the actual action of the signalling cascades. The parameters for these models were determined and optimised in a similar way using the FindSim experiment files and better optimised using HOSS optimisation.

This project ended with an attempt to find optimal parameters for the mass-action model by trying to optimise the K_d and tau values for the reaction binding mechanisms and K_m and K_{cat} for the enzyme reactions. The D3 and the D4 model can be combined together by using MOUSE to find better parameters. It uses the D3 model to generate synthetic experiments to describe the better detailing and fit required for the molecules in a D4 model. Furthermore, these models can be used to compare the concentration and production of these molecules during neurodegenerative diseases like Alzheimer's and Parkinson's. Those can be compared with the healthy model to check how the pathways are affected by the administration of chemical drugs and can be used for further medical implications.

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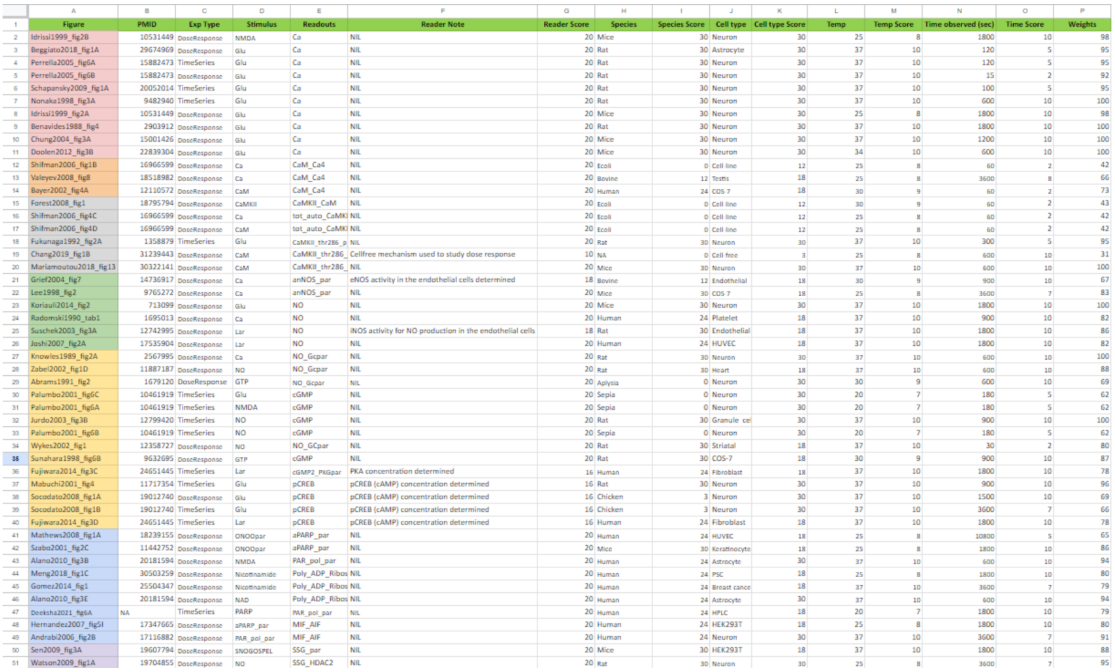
6 Supplementary Material

All the files and scripts (models, experiments, weight-sheet and config files) made throughout the course of the project have been uploaded on the lab's e-labnotes and belongs to the rights of Bhalla Lab, National Centre for Biological Sciences (NCBS), Bangalore, India. The experiment files created for optimising the parameters are also uploaded and available at FindSim Web.

Species		Temp		Cell type		Time Obs	
Mouse/Rat	1	37	1	Neuron	1	0-100	0.2
Human	0.8	30	0.9	Other	0.6	100-600	0.5
Bovine	0.4	25	0.8	Cell-line	0.5	600-1800	1
Chicken	0.1	10	0.6	Cellfree	0.1	1800-3600	0.8
NA	0	4	0.4			3600-	0.7

Figure 6: FindSim files experiments weights on the basis of following criteria for HOSS optimisation



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Figure	PMID	Exp Type	Stimulus	Readouts	Reader Note	Reader Score	Species	Species Score	Cell type	Cell type Score	Temp	Temp Score	Time observed (sec)	Time Score	Weights
2	Idrissi1999_fig2B	10531449	DoseResponse	NMDA	Ca	NIL	20	Mice	30	Neuron	30	25	8	1800	10	98
3	Beggiato2018_fig1A	29674969	DoseResponse	Glu	Ca	NIL	20	Rat	30	Astrocyte	30	37	10	120	5	95
4	Perrella2005_fig6A	15882473	TimeSeries	Glu	Ca	NIL	20	Rat	30	Neuron	30	37	10	120	5	95
5	Perrella2005_fig6B	15882473	DoseResponse	Glu	Ca	NIL	20	Rat	30	Neuron	30	37	10	15	2	92
6	Schapansky2009_fig1A	20052014	TimeSeries	Glu	Ca	NIL	20	Rat	30	Neuron	30	37	10	100	5	95
7	Nonaka1998_fig3A	9482940	TimeSeries	Glu	Ca	NIL	20	Rat	30	Neuron	30	37	10	600	10	100
8	Idrissi1999_fig2A	10531449	DoseResponse	Glu	Ca	NIL	20	Mice	30	Neuron	30	25	8	1800	10	98
9	Benavides1988_fig4	2903912	DoseResponse	Glu	Ca	NIL	20	Rat	30	Neuron	30	37	10	1800	10	100
10	Chung2004_fig3A	15001426	DoseResponse	Glu	Ca	NIL	20	Mice	30	Neuron	30	37	10	1200	10	100
11	Doolen2012_fig3B	22839304	DoseResponse	Glu	Ca	NIL	20	Mice	30	Neuron	30	34	10	600	10	100
12	Shifman2006_fig1B	16966599	DoseResponse	Ca	CaM_Ca4	NIL	20	Ecoli	0	Cell line	12	25	8	60	2	42
13	Valeyev2008_fig8	18518982	DoseResponse	Ca	CaM_Ca4	NIL	20	Bovine	12	Testis	18	25	8	3600	8	66
14	Bayer2002_fig4A	12110572	DoseResponse	CaM	CaM_Ca4	NIL	20	Human	24	COS-7	18	30	9	60	2	73
15	Forest2008_fig1	18795794	DoseResponse	CaMKII	CaMKII_CaM	NIL	20	Ecoli	0	Cell line	12	30	9	60	2	43
16	Shifman2006_fig4C	16966599	DoseResponse	Ca	tot_auto_CaMKI	NIL	20	Ecoli	0	Cell line	12	25	8	60	2	42
17	Shifman2006_fig4D	16966599	DoseResponse	CaM	tot_auto_CaMKI	NIL	20	Ecoli	0	Cell line	12	25	8	60	2	42
18	Fukunaga1992_fig2A	1358879	TimeSeries	Glu	CaMKII_thr286_p	NIL	20	Rat	30	Neuron	30	37	10	300	5	95
19	Chang2019_fig1B	31239443	DoseResponse	CaM	CaMKII_thr286_	Cellfree mechanism used to study dose response	10	NA	0	Cell free	3	25	8	600	10	31
20	Mariamoutou2018_fig13	30322141	DoseResponse	CaM	CaMKII_thr286_	NIL	20	Mice	30	Neuron	30	37	10	600	10	100
21	Grief2004_fig7	14736917	DoseResponse	Ca	anNOS_par	eNOS activity in the endothelial cells determined	18	Bovine	12	Endothelial	18	30	9	900	10	67
22	Lee1998_fig2	9765272	DoseResponse	Ca	anNOS_par	NIL	20	Mice	30	COS-7	18	25	8	3600	7	83
23	Koriauli2014_fig2	713099	DoseResponse	Glu	NO	NIL	20	Mice	30	Neuron	30	37	10	1800	10	100
24	Radomski1990_tab1	1695013	DoseResponse	Ca	NO	NIL	20	Human	24	Platelet	18	37	10	900	10	82
25	Suschek2003_fig3A	12742995	DoseResponse	Lar	NO	iNOS activity for NO production in the endothelial cells	18	Rat	30	Endothelial	18	37	10	1800	10	86
26	Joshi2007_fig2A	17535904	DoseResponse	Lar	NO	NIL	20	Human	24	HUVEC	18	37	10	1800	10	82
27	Knowles1989_fig2A	2567995	DoseResponse	Ca	NO_Gcpar	NIL	20	Rat	30	Neuron	30	37	10	600	10	100
28	Zabel2002_fig1D	11887187	DoseResponse	NO	NO_Gcpar	NIL	20	Rat	30	Heart	18	37	10	600	10	88
29	Abrams1991_fig2	1679120	DoseResponse	GTP	NO_Gcpar	NIL	20	Aplysia	0	Neuron	30	30	9	600	10	69
30	Palumbo2001_fig6C	10461919	TimeSeries	Glu	cGMP	NIL	20	Sepia	0	Neuron	30	20	7	180	5	62
31	Palumbo2001_fig6A	10461919	TimeSeries	NMDA	cGMP	NIL	20	Sepia	0	Neuron	30	20	7	180	5	62
32	Jurdo2003_fig3B	12799420	TimeSeries	NO	cGMP	NIL	20	Rat	30	Granule cel	30	37	10	900	10	100
33	Palumbo2001_fig6B	10461919	TimeSeries	NO	cGMP	NIL	20	Sepia	0	Neuron	30	20	7	180	5	62
34	Wykes2002_fig1	12358727	DoseResponse	NO	NO_GCpar	NIL	20	Rat	30	Striatal	18	37	10	30	2	80
35	Sunahara1998_fig6B	9632695	DoseResponse	GTP	cGMP	NIL	20	Rat	30	COS-7	18	30	9	900	10	87
36	Fujiwara2014_fig3C	24651445	TimeSeries	Lar	cGMP2_PKGpar	PKA concentration determined	16	Human	24	Fibroblast	18	37	10	1800	10	78
37	Mabuchi2001_fig4	11717354	TimeSeries	Glu	pCREB	pCREB (cAMP) concentration determined	16	Rat	30	Neuron	30	37	10	900	10	96
38	Socodato2008_fig1A	19012740	DoseResponse	Glu	pCREB	pCREB (cAMP) concentration determined	16	Chicken	3	Neuron	30	37	10	1500	10	69
39	Socodato2008_fig1B	19012740	TimeSeries	Glu	pCREB	pCREB (cAMP) concentration determined	16	Chicken	3	Neuron	30	37	10	3600	7	66
40	Fujiwara2014_fig3D	24651445	TimeSeries	Lar	pCREB	pCREB (cAMP) concentration determined	16	Human	24	Fibroblast	18	37	10	1800	10	78
41	Mathews2008_fig1A	18239155	DoseResponse	ONOOpar	aPARP_par	NIL	20	Human	24	HUVEC	18	25	8	10800	5	65
42	Szabo2001_fig2C	11442752	DoseResponse	ONOOpar	aPARP_par	NIL	20	Mice	30	Keratinocyte	18	25	8	1800	10	86
43	Alano2010_fig3B	20181594	DoseResponse	NMDA	PAR_pol_par	NIL	20	Human	24	Astrocyte	30	37	10	600	10	94
44	Meng2018_fig1C	30503259	DoseResponse	Nicotinamide	Poly_ADP_Ribos	NIL	20	Human	24	PSC	18	25	8	1800	10	80
45	Gomez2014_fig1	25504347	DoseResponse	Nicotinamide	Poly_ADP_Ribos	NIL	20	Human	24	Breast cance	18	37	10	3600	7	79
46	Alano2010_fig3E	20181594	DoseResponse	NAD	Poly_ADP_Ribos	NIL	20	Human	24	Astrocyte	30	37	10	600	10	94
47	Deeksha2021_fig6A	NA	TimeSeries	PARP	PAR_pol_par	NIL	20	Human	24	HPLC	18	20	7	1800	10	79
48	Hernandez2007_fig5I	17347665	DoseResponse	aPARP_par	MIF_AIF	NIL	20	Human	24	HEK293T	18	25	8	1800	10	80
49	Andrabi2006_fig2B	17116882	DoseResponse	PAR_pol_par	MIF_AIF	NIL	20	Human	24	Neuron	30	37	10	3600	7	91
50	Sen2009_fig3A	19607794	DoseResponse	SNOGOSPEL	SSG_par	NIL	20	Mice	30	HEK293T	18	37	10	1800	10	88
51	Watson2009_fig1A	19704855	DoseResponse	NO	SSG_HDAC2	NIL	20	Rat	30	Neuron	30	25	8	3600	7	95

Figure 7: FindSim files experiments scores on the basis of above criteria for HOSS optimisation

References

[AHP07] Antonio Alcaro, Robert Huber, and Jaak Panksepp. Behavioral functions of the mesolimbic dopaminergic system: An affective neuroethological perspective. *Brain Research Reviews*, 56:283–321, 2007.

[Ben11] Eduardo E. Benarroch. Nitric oxide. *Neurology*, 77(16):1568–1576, 2011.

[Bha15] Upinder S Bhalla. Multiscale object-oriented simulation environment, 2015.

[Bha21a] Upinder S Bhalla. Hierarchical optimization of systems simulations: A suite of tools working with the findsim project for model optimization, 2021.

[Bha21b] Upinder S. Bhalla. Hilltau: A fast, compact abstraction for model reduction in biochemical signaling networks. *PLOS Computational Biology*, 17(11):1–31, 11 2021.

[HDF13] Neil Hardingham, James Dachtler, and Kevin Fox. The role of nitric oxide in pre-synaptic plasticity and homeostasis. *Frontiers in Cellular Neuroscience*, 7, 2013.

[HRB13] G. V. Harsha Rani and Upinder S. Bhalla. Database of quantitative cellular signaling (doqcs). pages 534–537, 2013.

[HZ14] Yao Hu and Dong-Ya Zhu. Chapter six - hippocampus and nitric oxide. In Gerald Litwack, editor, *Nitric Oxide*, volume 96 of *Vitamins Hormones*, pages 127–160. Academic Press, 2014.

[iOL15] Shu ichi Okamoto and Stuart A. Lipton. S-nitrosylation in neurogenesis and neuronal development. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1850(8):1588–1593, 2015. Redox regulation of differentiation and de-differentiation.

[Juh98] JH Juhl. Fibromyalgia and the serotonin pathway. *Alternative medicine review : a journal of clinical therapeutic*, 3(5):367—375, October 1998.

[KSF21] Conny Kopp-Scheinpflug and Ian D. Forsythe. Nitric oxide signaling in the auditory pathway. *Frontiers in Neural Circuits*, 15, 2021.

[KSJ91] Eric R. Kandel, James H. Schwartz, and Thomas M. Jessell, editors. *Principles of Neural Science*. Elsevier, New York, third edition, 1991.

[Pow94] M. J. D. Powell. *A Direct Search Optimization Method That Models the Objective and Constraint Functions by Linear Interpolation*, pages 51–67. Springer Netherlands, Dordrecht, 1994.

[PPGBM19] Pol Picón-Pagès, Joan Garcia-Buendia, and Francisco J. Muñoz. Functions and dysfunctions of nitric oxide in brain. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1865(8):1949–1967, 2019. Post-Translational Modifications In Brain Health And Disease.

[TSWG11] Irmgard Tegeder, Reynir Scheving, Ilka Wittig, and Gerd Geisslinger. Sno-ing at the nociceptive synapse? *Pharmacological Reviews*, 63(2):366–389, 2011.

[VHSB18] Nisha A. Viswan, Gubbi Vani HarshaRani, Melanie I. Stefan, and Upinder S. Bhalla. Findsim: A framework for integrating neuronal data and signaling models. *Frontiers in Neuroinformatics*, 12, 2018.