iGEM MIT_MAHE
Model Handbook

Acknowledgements

The curation of this handbook by Varun Udaya Kumar, Vaishnavi Chavan and Adithi Somayaji would not have been possible without the exceptional support of Dr S. Balaji. His enthusiasm and knowledge kept the information on track from start to end. Soumodeep Sarkar, our fellow teammate, has also given us insight into the documentation. We thank Varun Udaya Kumar for MATLAB modelling, writing, editing and research, Vaishnavi Chavan for VMD model and writing, Beejady Adithi Somayaji for writing and editing. The expertise and skills of everyone involved has improved this handbook in innumerable ways.

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1. Introduction

Modelling is an integral part of biological analysis. Two types of modelling have been used for analysis.

Mathematical model:

MATLAB is a programming platform designed specifically for engineers and scientists. The heart of MATLAB is the MATLAB language, a matrix-based language allowing the most natural expression of computational mathematics.

Using MATLAB, you can:

1. Analyse data
2. Develop algorithms
3. Create models and applications

We have used MATLAB - SimBiology to simulate and analyse our biological systems. Two different models have been made.

VMD model:

Since the 1950s, Molecular dynamics is used to understand the interaction between N bodies in a system, a problem which dated back to 15th century undertaken by Newton. Indeed, at their core, MD software are programs written to calculate Newton's laws of motion acting on a system of interacting particles.

Molecular dynamics is an indispensable research tool, whether it is used as a visual aid or as a way to study conformational changes in proteins and substrate/inhibitor action on proteins. In times like the COVID19 pandemic, when in vivo and in vitro studies are not feasible in labs, MD becomes an invaluable tool to aid the progress of work. It finds applications in fields like material science, biochemistry, biophysics etc.

In recent years, increase in computing performance (like the use of GPUs) complimented the improvements made in the optimization of MD algorithms. Molecular dynamics simulation will play a huge role in the future, as with the availability of superior computing power at lower costs everywhere it will become easier for both students and professionals to conduct increasingly accurate studies of intricate molecular systems.

We have modelled our two enzymes encoded by MerA and MerB gene on VMD to visualize the interactions between the mercury and the cysteine residues.

2. Mathematical Model

A. Model A:

Aim: This model describes the activity of our dual enzyme system

Principle:

MerB encodes the Alkylmercury lyase enzyme and is usually found immediately downstream to MerA. It catalyzes breaking the bond between carbon and mercury through the protonolysis of compounds such as methylmercury. This produces the less mobile Hg (II) which is then reduced to Hg (0) by MerA.

Model:

In order to assess the order of concentration of enzymes required for optimal conversion, we have designed an experiment (refer to iGEM MIT_MAHE Composite BioBrick 1 handbook for complete protocol). This experiment would provide three graphs

- Graph of mercury concentration vs time is obtained for constant concentration of enzyme
- Graph of enzyme concentration vs activity is obtained.
- Graph of no of cells vs enzyme concentration graph is obtained.

Using the graphs we can estimate the optimal amount and enzyme required to be produced for optimal activity and thus, estimate the optimum CFU/mL to be used to get maximum activity.

To provide a range of concentration of enzyme that can be used in order to attain the first graph, we have designed a MATLAB model.

We have used Michaelis Menten kinetics to simulate the kinetics of our two enzymes alkylmercury lyase and mercuric reductase.

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \overset{k_2}{\Leftrightarrow} E + P$$

Eq1: Michaelis Menten kinetics-based enzyme reaction mechanism

The following ODEs are used to describe such a mechanism:

$$\frac{ds}{dt} = -k_1.E.S + k_{-1}.E.S$$

Equation-2

$$\frac{dES}{dt} = k_1E.S - (k_{-1} + k_2)ES$$

Equation-3

$$\frac{dE}{dt} = -k_1E.S + (k_{-1} + k_2)ES$$

Equation-4

$$\frac{dP}{dt} = k_2ES$$

Equation-5

The main objective of such a description is to find the rate at which the product is produced. Hence, the above ODEs have to be solved w.r.t. $\frac{dp}{dt}$

Assumptions to be considered:

1. ES formation is much faster than E + P formation. → k_1, k_{-1} >>> k_2
2. Steady state condition → $\frac{dES}{dt} = 0$ (ES concentration is constant)

Using the above conditions and solving the ODE, we get

$$\frac{dP}{dt} = \frac{k_1E_{total}S}{k_1S + k_{-1} + k_2}$$

Equation-6

where $E_{total} = ES + E$

$$\frac{dP}{dt} = \frac{V_{max}[S]}{[S] + K_m}$$

Equaton-7

where $V_{max} = k_2 \times E_{total}$ and $K_m = \frac{k_{-1} + k_2}{k_1}$

Moreover, the rate of product formation is equal to the rate of substrate utilization $\left(\frac{-ds}{dt}\right)$

Therefore,

$$\frac{-ds}{dt} = \frac{dP}{dt}$$

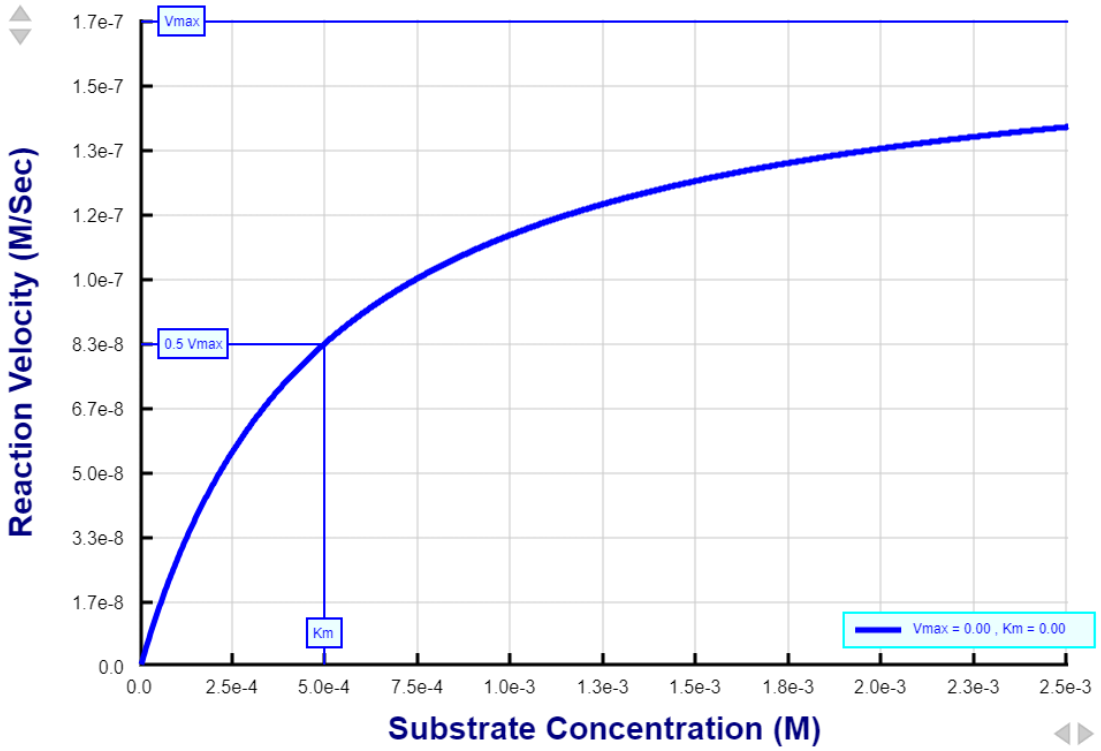
Equation-8

$$\frac{-ds}{dt} = \frac{V_{max}S}{S + K_m}$$

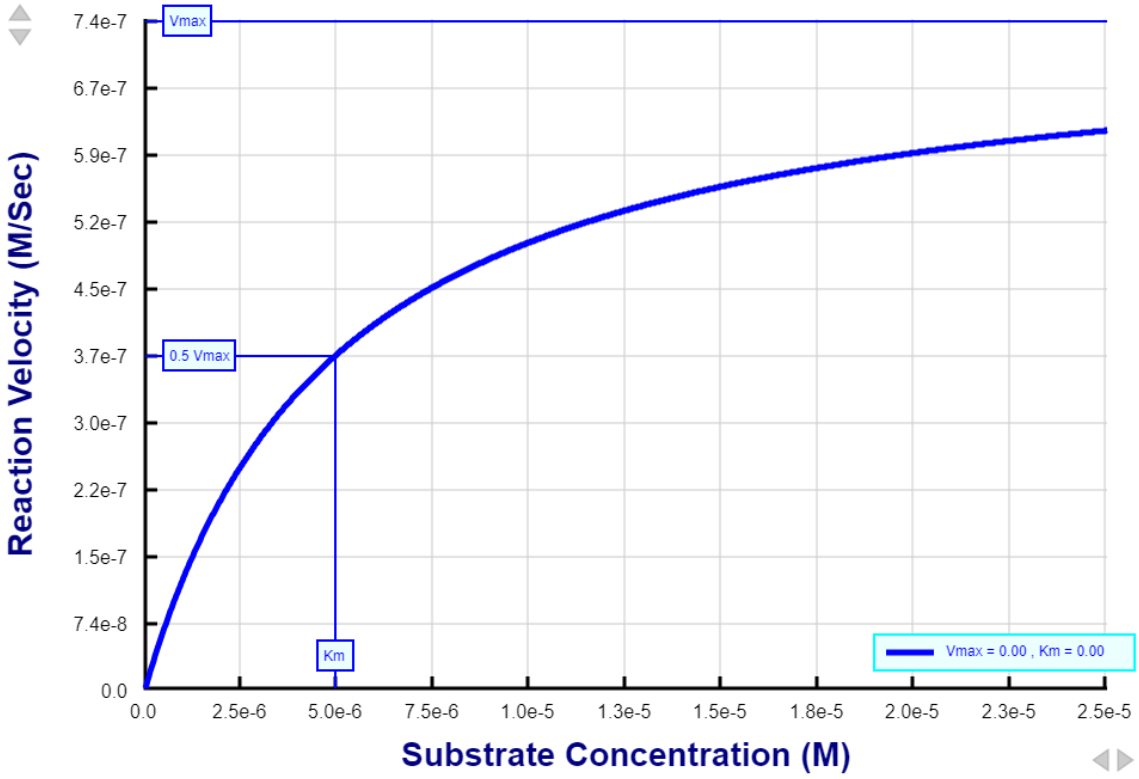
Equation-9

$$\frac{ds}{dt} = \frac{-V_{max}S}{S + K_m}$$

Equation-10



Michaelis-Menten curve for MerA



Michaelis-Menten curve for MerB

Equations:

Fluxes

$$Reaction\ 1 = \left(\frac{V_{m1}.CH_3Hg}{K_{m1} + CH_3Hg}\right)$$

Equation-11

$$\text{Reaction } 2 = \left(\frac{V_{m2}.[Hg^{2+}]}{K_{m2} + [Hg^{2+}]}\right)$$

Equation-12

Here,

- K_{m1} value for alkylmercury lyase is **0.5 mM.**
- K_{m2} value for mercuric reductase is **0.0005mM.**
- K_{cat1} value for alkylmercury lyase is **0.0167/s.**
- K_{cat2} value for mercuric reductase is **0.0743/s.**

ODES

$$\frac{dCH_3Hg}{dt} = \left(-\left(\frac{V_{m1}.[CH_3Hg]}{K_{m1} + [CH_3Hg]}\right)\right)$$

Equation-13

$$\frac{d[Hg^{2+}]}{dt} = \left(\left(\frac{V_{m1}.[CH_3Hg]}{K_{m1} + [CH_3Hg]}\right) - \left(\frac{V_{m2}.[Hg^{2+}]}{K_{m2} + [Hg^{2+}]}\right)\right)$$

Equation-14

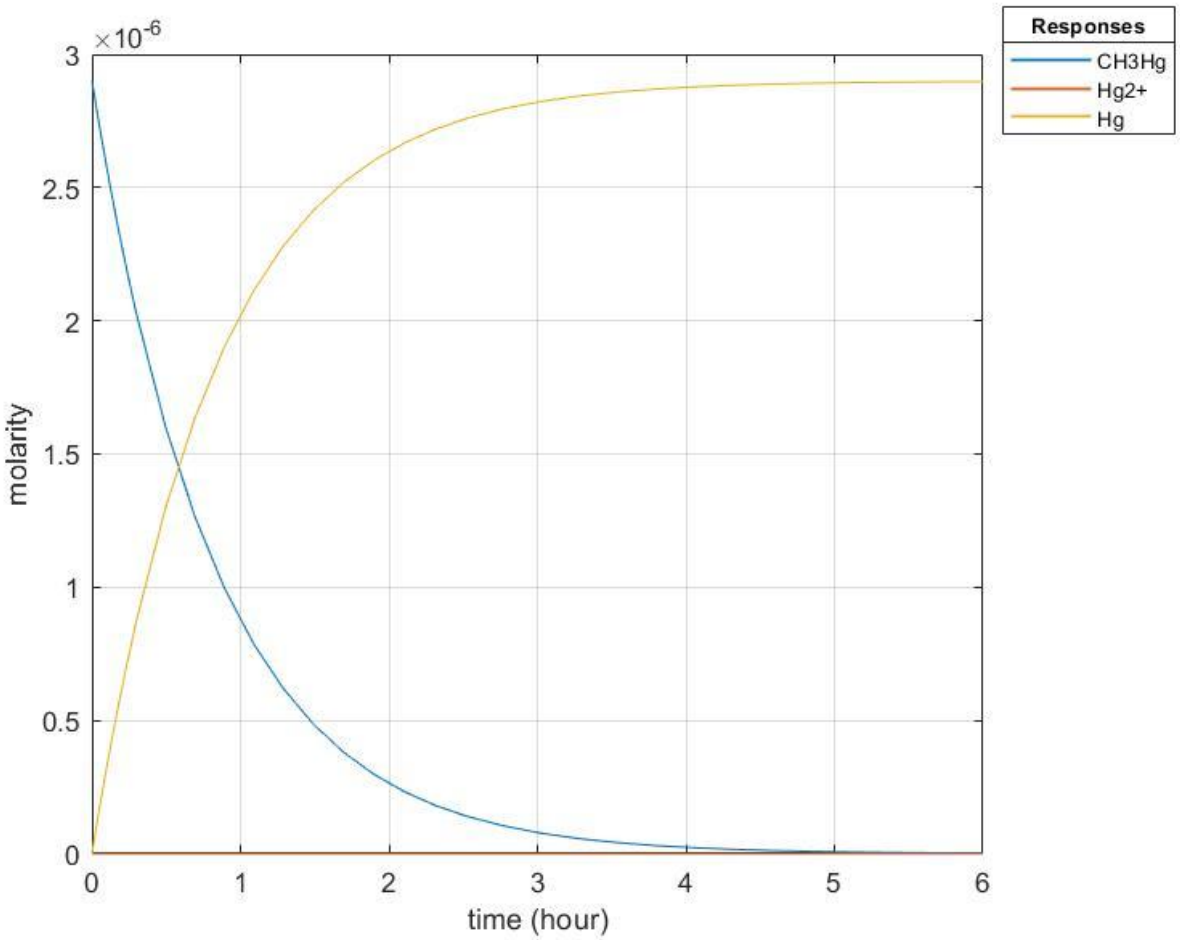
$$\frac{dHg}{dt} = \left(\frac{V_{m2}.[Hg^{2+}]}{K_{m2} + [Hg^{2+}]}\right)$$

Equation-15

Observations:

We have considered the optimal time range to be 6-7 hours as the retention time of food in the small intestine is about the same.

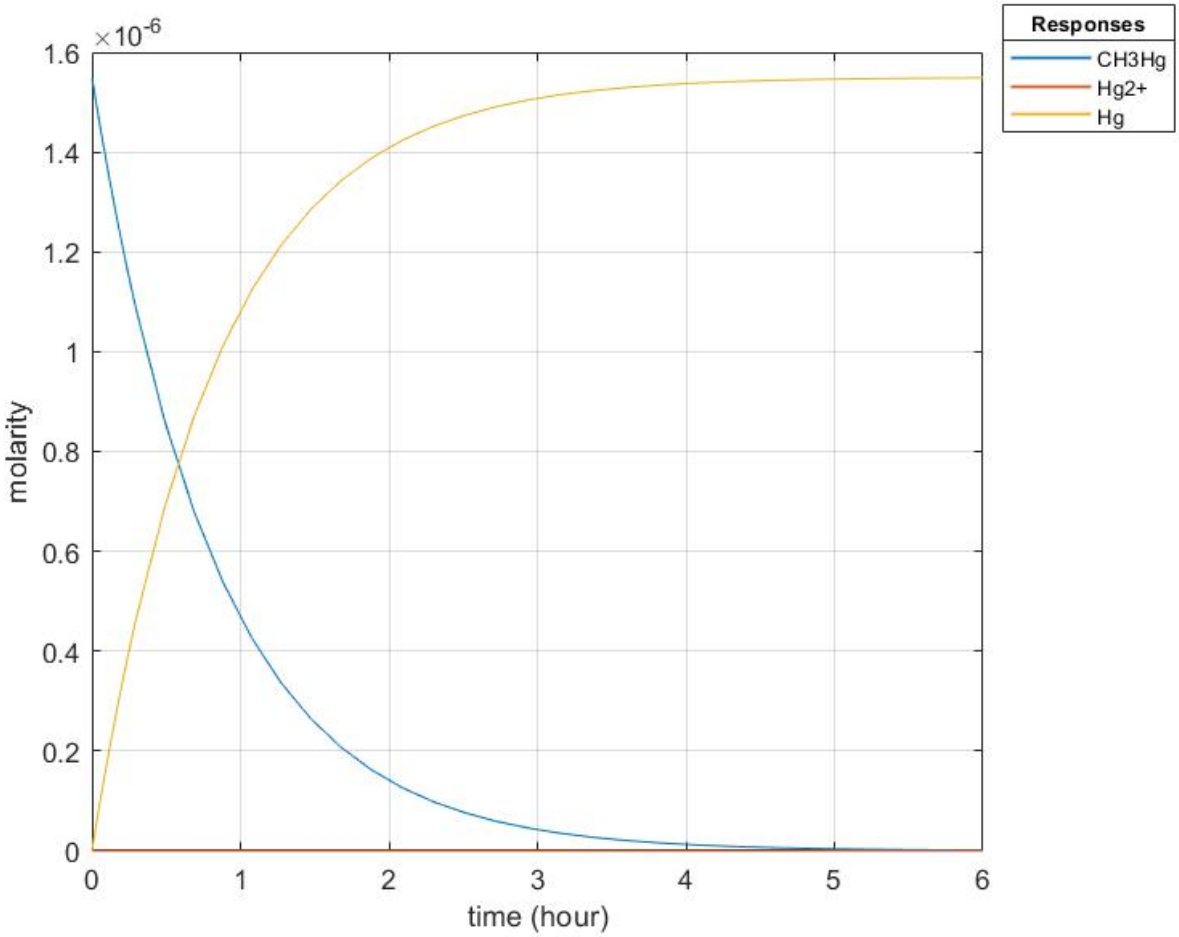
Concentration of enzyme used is 10^{-4}.



Graph for the activity for MerA and MerB at [CH3Hg] =2.9 μM (Mercury content in king mackerel)

Observation:

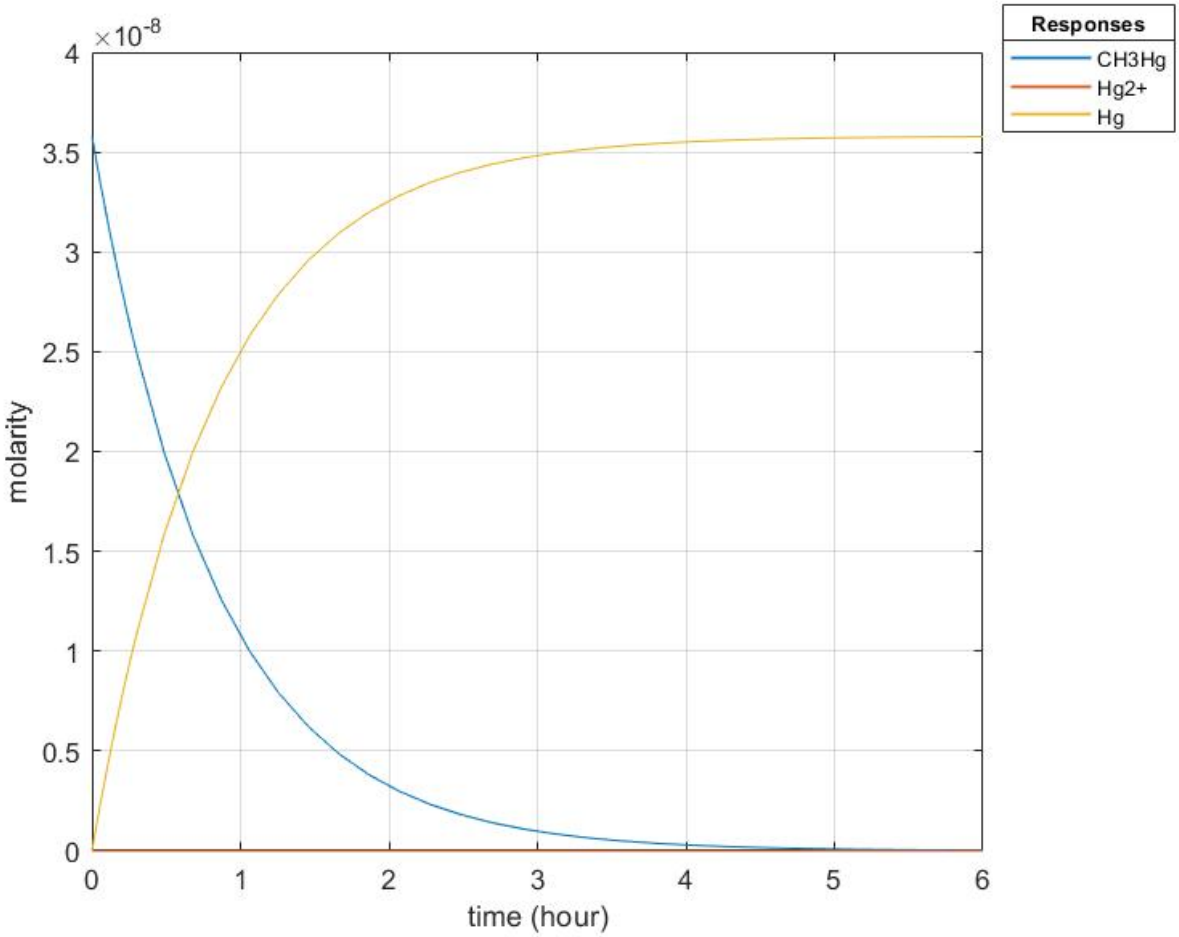
At relatively high concentrations of methylmercury, the conversion rate rapidly increases. The time taken for complete conversion is optimal.



Graph for the activity for MerA and MerB at [CH_3Hg] =1.55 µM (Mercury content in tuna fish)

Observation:

At moderate concentration of mercury and same concentration of enzyme, the time required for complete conversion remains the same.

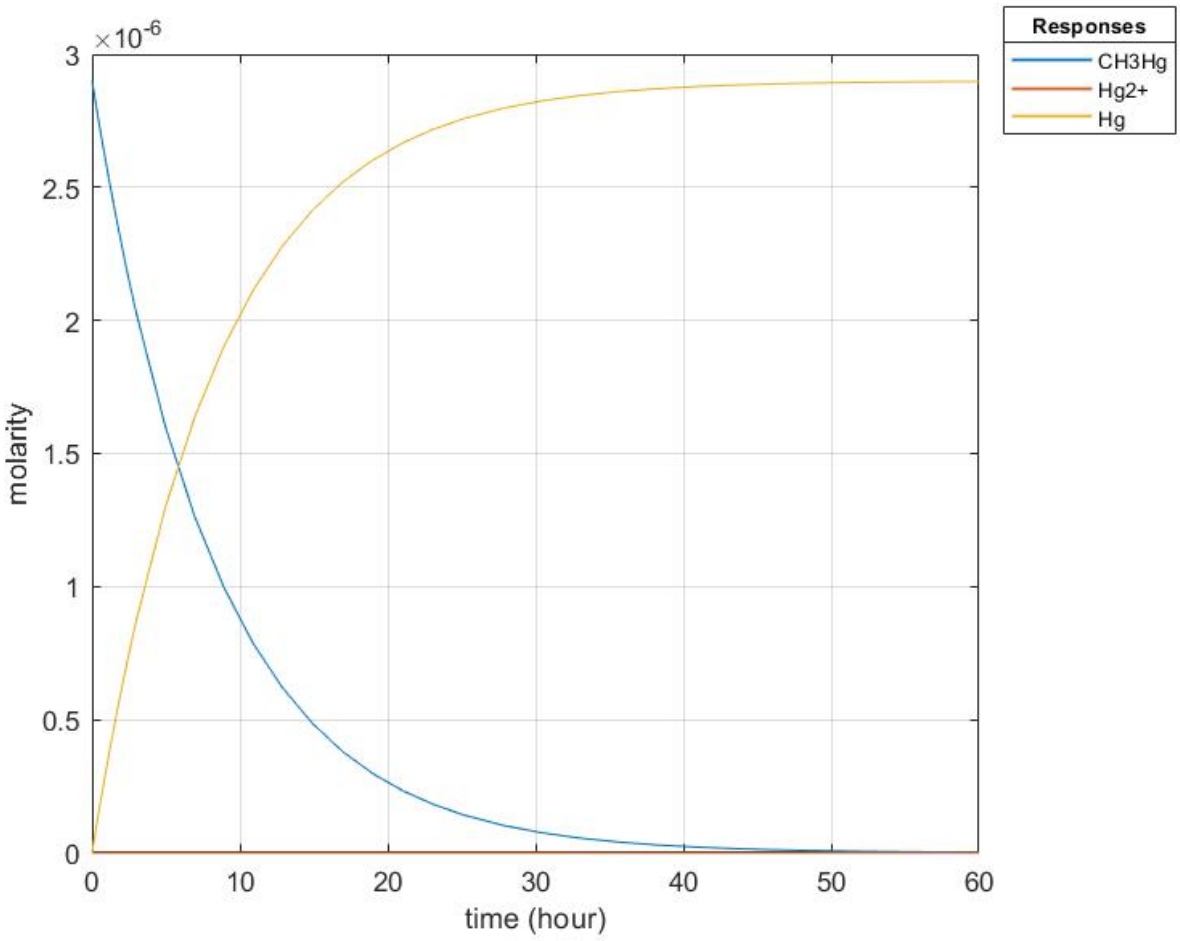


Graph for the activity for MerA and MerB at [CH_3Hg] = 0.0358nM (Mercury content in shrimp)

Observation:

At low concentrations of methylmercury and same enzyme concentrations, the time taken for complete conversion remains approximately the same and is optimal.

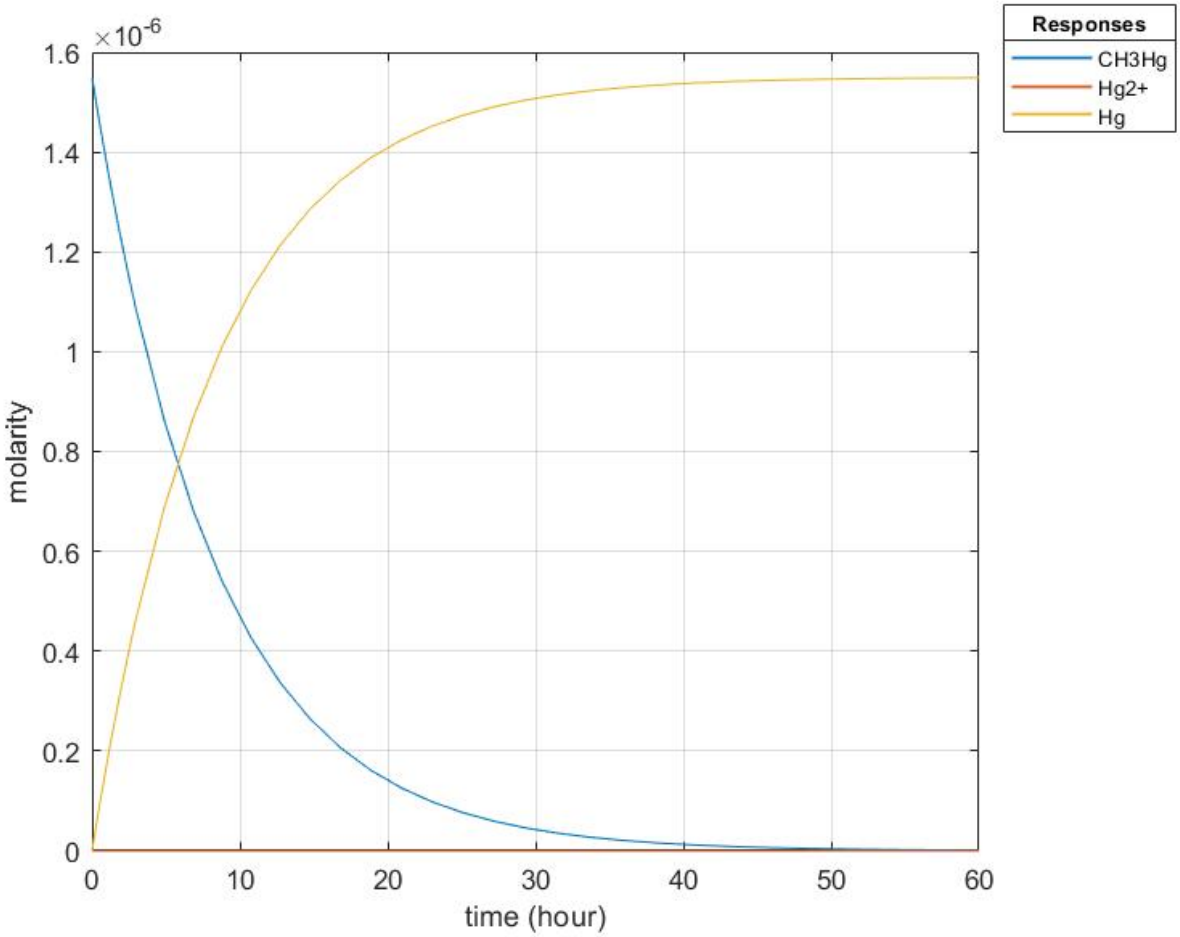
Enzyme concentration of 10^{-5}



Graph for the activity for MerA and MerB at [CH3Hg] =2.9 µM (Mercury content in king mackerel)

Observation:

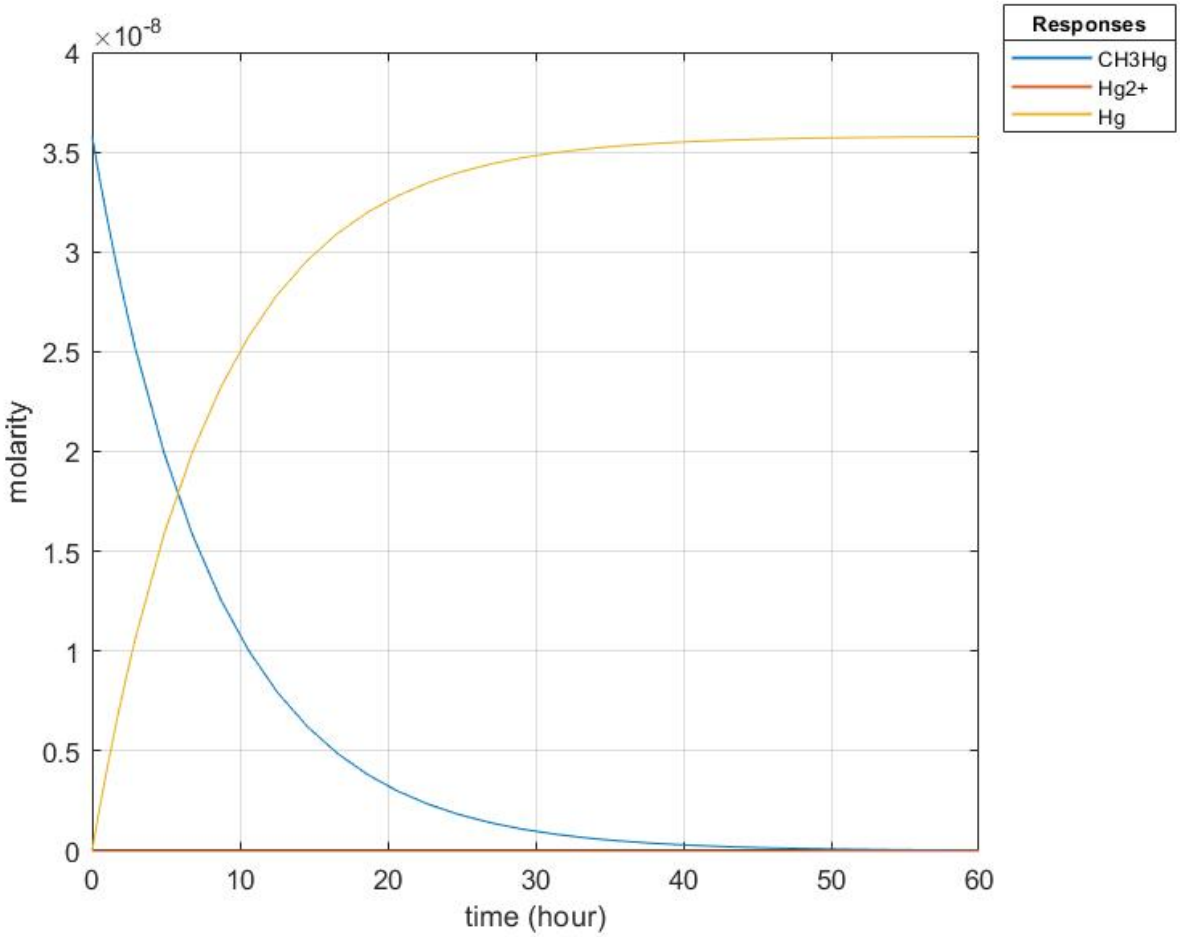
At high concentrations, the time taken for complete conversion exceeds the optimal time range decided, taking much longer.



Graph for the activity for MerA and MerB at [CH_3Hg] =1.55 µM (Mercury content in tuna fish)

Observation:

At moderate concentrations, the time taken for complete conversion exceeds the optimal time range decided, taking much longer.

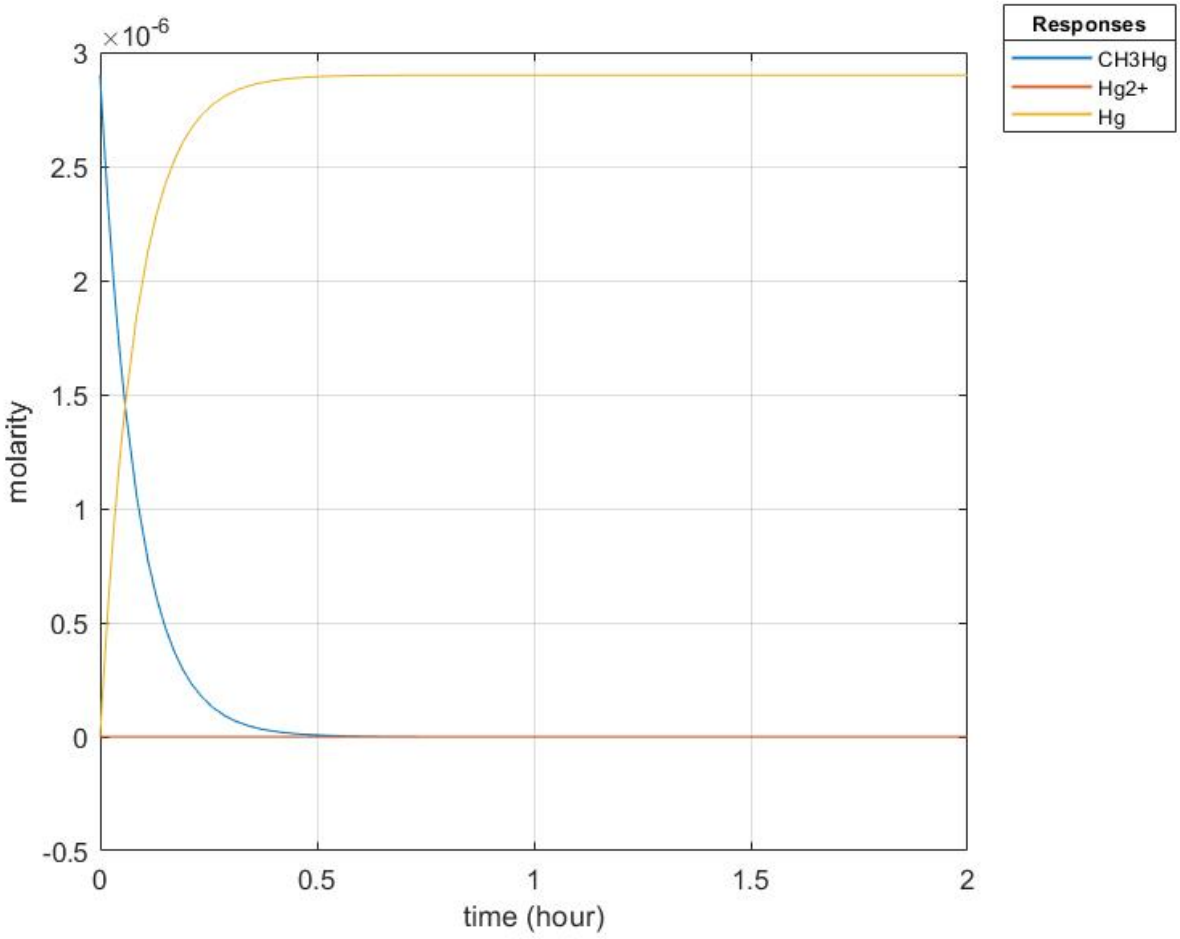


Graph for the activity for MerA and MerB at [CH_3Hg] = 0.0358nM (Mercury content in shrimp)

Observation:

At low concentrations, the time taken for complete conversion exceeds the optimal time range decided, taking much longer.

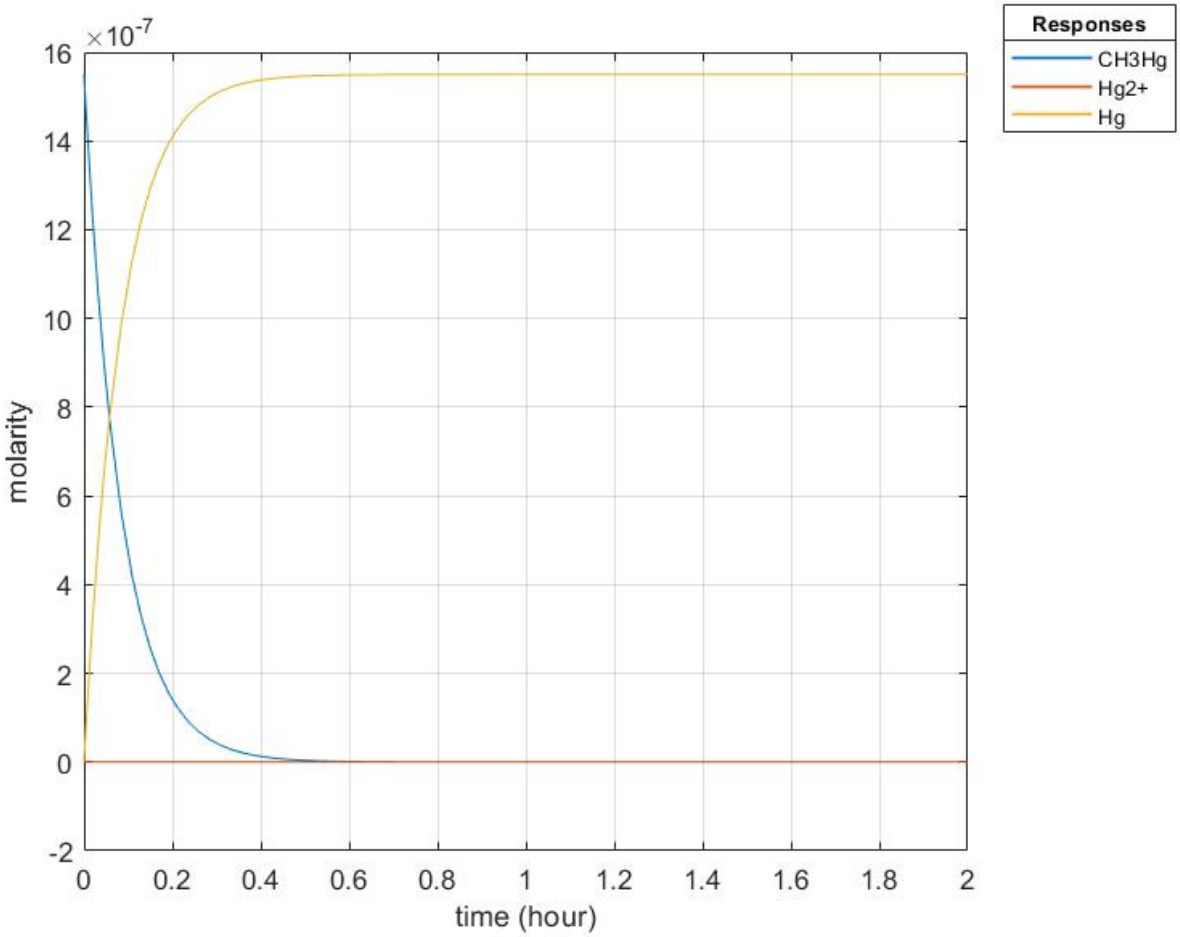
Enzyme concentration of 10^{-3}



Graph for the activity for MerA and MerB at [CH3Hg] =2.9 µM (Mercury content in king mackerel)

Observation:

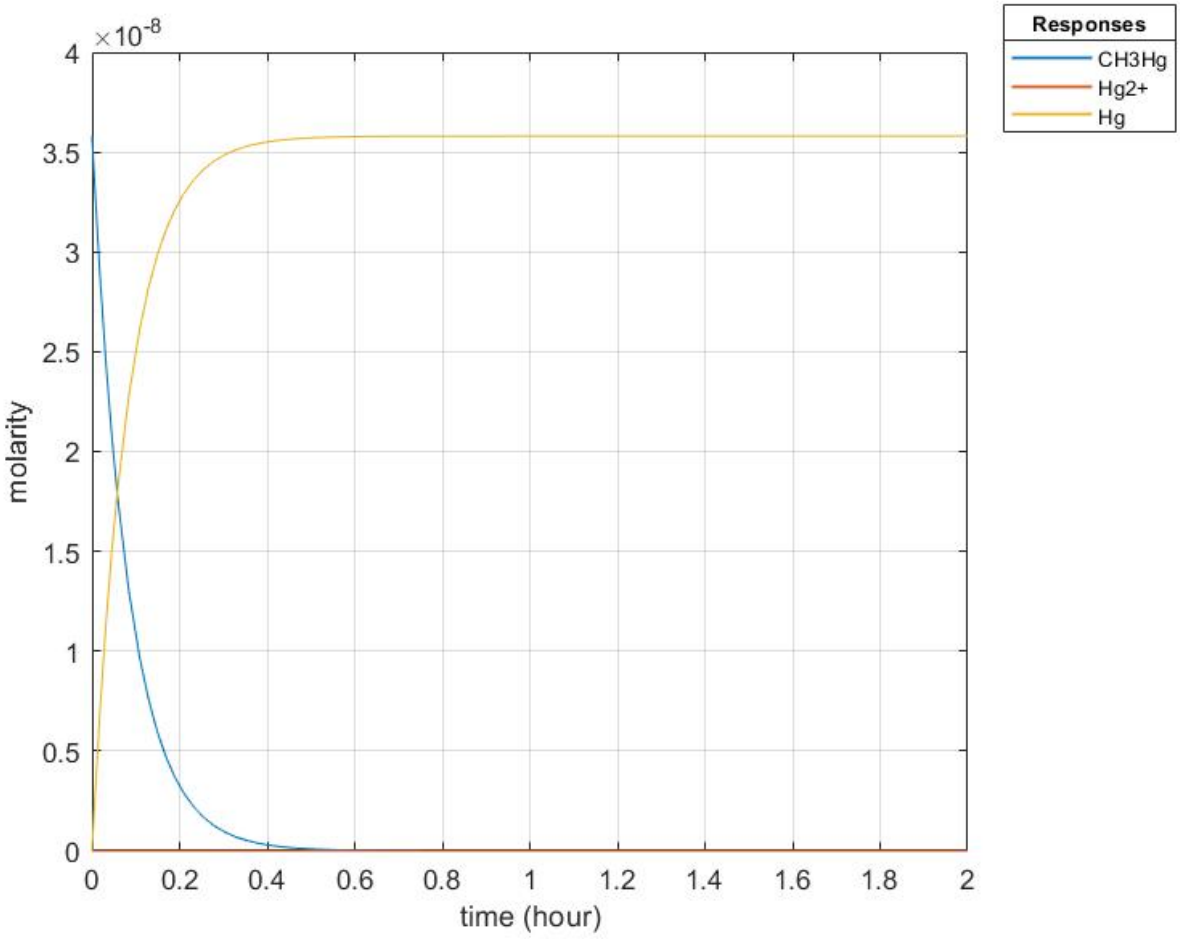
At high concentrations, the time taken for complete conversion is in the optimal time range decided. However, increased concentration of enzyme consumes a lot of energy.



Graph for the activity for MerA and MerB at [CH_3Hg] =1.55 µM (Mercury content in tuna fish)

Observation:

At high concentrations, the time taken for complete conversion is in the optimal time range decided. However, increased concentration of enzyme consumes a lot of energy.



Graph for the activity for MerA and MerB at [CH_3Hg] = 0.0358nM (Mercury content in shrimp)

Observation:

At high concentrations, the time taken for complete conversion is in the optimal time range decided. However, increased concentration of enzyme consumes a lot of energy.

Results:

We have chosen the optimal order of enzyme concentration to be 10^{-4} as the reduction rate falls within our time range. However, more experiments are to be performed to see whether 10^{-3} concentration can be utilized.

B. Model B

Aim: To describe the activity of the transport system.

Principle:

MerT, MerP, MerC and MerE are proposed as our transport genes. MerP is the periplasmic component of the mer transport system which helps in the uptake of mercury inside the cell. MerT is a transmembrane protein which receives mercury from MerP, at its first transmembrane helix and transports it into the cytoplasm of the bacterial cell. MerC is a transmembrane component of the mer transport system which helps in the uptake of mercury inside the cell. It can function without the help of MerP. MerE is a transmembrane component of the mer transport system which helps in the uptake of mercury inside the cell. It helps in the transport of organo-mercury compounds.

Model:

Assumption

- The production of the proteins has already started due to a small amount of CH_3Hg having diffused inside the cell priorly and bound with the MerR, initiating the transcription.
- Amount of protein within the cell will remain constant.

Equations:

Chemical equations being modelled:

$$CH_3Hg_{out} + MerC \Leftrightarrow CH_3Hg_{in} + MerC, (k_{f_1}, k_{r_1})$$

Equation-1

$$CH_3Hg_{out} + MerP \Leftrightarrow MerP - CH_3Hg\ Complex, (k_{f_2}, k_{r_2})$$

Equation-2

$$MerP - CH_3Hg\ Complex + MerT \Leftrightarrow MerT - CH_3Hg\ Complex + MerP \quad (k_{f_3}, k_{r_3})$$

Equation-3

$$MerP - CH_3Hg\ Complex + MerE \Leftrightarrow\ MerE - CH_3Hg\ Complex + MerP\ (k_{f_4}, k_{r_4})$$

Equation-4

$$MerT - CH_3Hg\ Complex \Leftrightarrow\ MerT + CH_3Hg_{in}\ (k_{f_5}, k_{r_5})$$

Equation-5

$$MerE - CH_3Hg\ Complex \Leftrightarrow\ MerE + CH_3Hg_{in}\ (k_{f_6}, k_{r_6})$$

Equation-6

Fluxes:

$$reaction\_1 = k_{f_1} \times [CH_3Hg_{out}] \times MerC - k_{r_1}[CH_3Hg_{in}] \times MerC$$

$$reaction\_2 = k_{f_2} \times [CH_3Hg_{out}] \times MerP - k_{r_2}[MerP - CH_3Hg\ complex]$$

$$reaction\_3 = k_{f_3}[MerP - CH_3Hg\ complex] \times MerT - k_{r_3}[MerT - CH_3Hg\ complex] \times MerP$$

$$reaction\_4 = k_{f_4}[MerP - CH_3Hg\ complex] \times MerE - k_{r_4}[MerE - CH_3Hg\ complex] \times MerP$$

$$reaction\_5 = k_{f_5} \times [MerT - CH_3Hg\ complex]$$

$$reaction\_6 = k_{f_6} \times [MerE - CH_3Hg\ complex]$$

ODEs:

$$\frac{d([CH_3Hg\ out])}{dt} = (-reaction\_1 - reaction\_2)$$

$$\frac{d([CH_3Hg\ in])}{dt} = (reaction\_1 + reaction\_5 + reaction\_6)$$

$$\frac{d(MerP)}{dt} = (-reaction\_2 + reaction\_3 + reaction\_4)$$

$$\frac{d([MerP - CH_3Hg\ complex])}{dt} = (reaction\_2 - reaction\_3 - reaction\_4)$$

$$\frac{d(MerT)}{dt} = (-reaction\_3 \ + reaction\_5)$$

$$\frac{d(MerE)}{dt} = (-reaction\_4 \ + reaction\_6)$$

$$\frac{d([MerT-CH_3Hg\ complex])}{dt} = (reaction\_3 \ - reaction\_5)$$

$$\frac{d([MerE-CH_3Hg\ complex])}{dt} = (reaction\_4 \ - reaction\_6)$$

Results:

Solving the ODE for CH3Hg in and CH3Hg out, we will get,

CH_3Hg_{out} = (MerP-CH_3Hg x k_{r2} - C_1 x exp(-t x (MerC x k_{f1} + MerP x k_{f2})) + $CH3Hg_{in}$ x MerC x k_{r1})/(MerC x k_{f1} + MerP x k_{f2})

CH_3Hg_{in} = (MerE - CH_3Hg x k_{f6} + MerT - CH_3Hg x k_{f5} - C_1 x exp(-MerC x k_{r1} x t) + CH_3Hg_{out} x MerC x k_{f1})/(MerC x k_{r1})

3. VMD MODEL

Introduction:

Visual molecular dynamics is a molecular visualization program used for display, animation, and analysis of large biomolecular systems using 3-D graphics and built-in scripting.

VMD was used to model the different enzymes used by *Escherichia Coli Nissle 1917* to convert organometallic compounds into elemental mercury.

We used CHARMM force-field with additional entries in the topology and parameter files for the mercury ion and cysteine (sulphur) interactions.

Proteins being modelled:

Alkylmercury lyase – It is encoded by MerB and plays a major role in breaking the bond between Hg and carbon atom from an organic radical releasing Hg (II) to be reduced by MerA. The purified protein requires a minimum two-fold molar excess of thiol over alkylmercury substrate to exhibit any activity and prefers the physiological thiol cysteine to non-physiological mercaptans. The enzyme has a very broad substrate tolerance, handling both alkyl and aryl mercurials, with a slight preference for the latter.

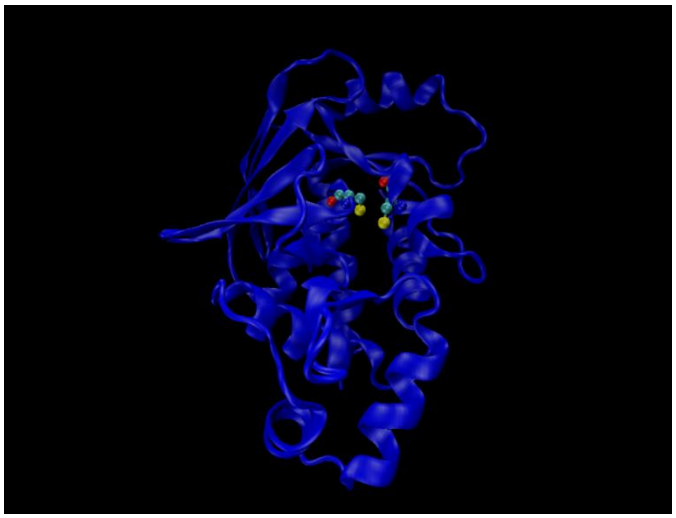


Fig 1: Alkylmercury Lyase without organic mercury. New cartoons representation with residues highlighted in CPK

Mercuric reductase – It is encoded by MerA and presents itself as a homodimer. It has two terminal sites containing a pair of cysteines, short C terminal and a long N terminal. C terminal cysteine is responsible for catching Hg (II) ions from solution and delivering them to the active site whereas the pair of inner cysteines are responsible for establishing chemical bonds with mercury.

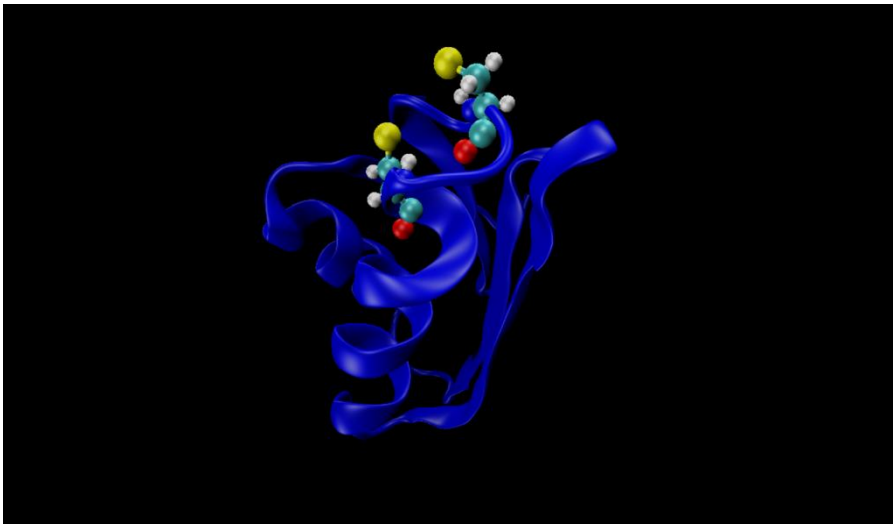


Fig 3: Mercuric reductase without mercury. New cartoons representation with residues highlighted in CPK

Model:

Alkylmercury Lyase:

This protein (MerB) cleaves the bond between the carbon and mercury ion in the organometallic compound and transfers the resulting mercury ion (Hg^{2+}) to mercuric reductase (MerA).

The active site of this protein is CYS95 and CYS 158. Here we show only one chain of the dimer of the MerB complex.

Searches for PDB files of MerB with an organometallic ligand were futile, therefore a carbon atom was inserted into the PDB file manually after calculating the distance between mercury and carbon in methyl mercury compound.

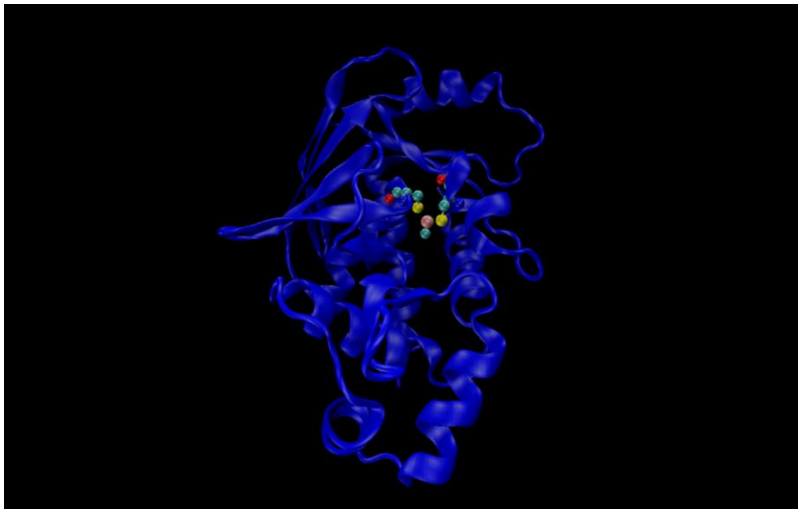


Fig 5: Alkylmercury Lyase with organic mercury. New cartoons representation with residues highlighted in CPK

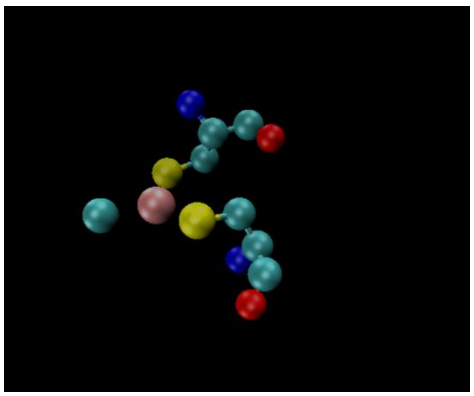


Fig 6: Cysteine residue with mercury. Cysteine residues represented in CPK.

Mercuric Reductase:

Mercuric Reductase, as the name implies, reduces the mercury ion (Hg^{2+}) to elemental mercury (Hg). The binding site for this protein is CYS10 and CYS 13.

We observe that the active sites for both proteins involve cysteine residues. It was inferred that the S-Hg-S Bridge plays an important role in the cleavage and reduction reactions.

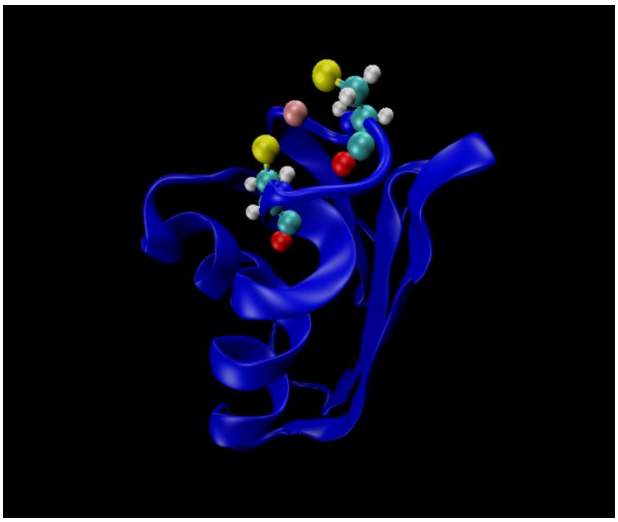


Fig 7: Mercuric reductase with mercury. New cartoons representation with residues highlighted in CPK

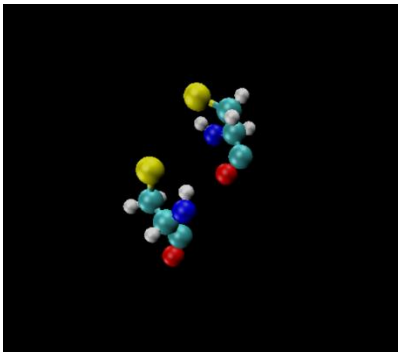


Fig 8: Cysteine residue without mercury. Cysteine residues represented in CPK.

Challenges and issues faced:

The final version of each molecule presented was obtained after making different small changes and testing it repeatedly. Those changes varied from changing the 3-letter code of mercury everywhere in the PDB file to manually inserting a carbon atom to make methyl mercury.

The most difficult hurdle that we faced was that the CHARMM files do not have information on mercury ions, we were forced to refer to a 2006 paper which gave us the required information. We tweaked top_all27_prot_lipid_na.inp (a CHARMM topology file) multiple times over the course of a few weeks to no success. We also reached out to the authors of the previously mentioned 2006 paper and another paper which referenced it to gain some insight into the problem but unfortunately, we didn't receive any reply.

Our aim was to show the stability of our enzyme by determining Root Mean Square Deviation of the protein-ligand system. We failed to do so due to the uncertainty about mercury ion properties. The results we obtained at the time of submission were too unsatisfactory for us to proceed with the analysis.

We are grateful for the help and advice Dr S. Balaji, a professor from the Biotechnology department has given us. On several occasions his remarks and guidance have removed some difficulty we were facing. When we asked about how to insert a ligand in the specific binding site, he gave us a list of options that included "merge the coordinates of the ligand manually and try", "docking the ligand" and "you can simply edit the mercury by appending a methyl group".

4. GLOSSARY

Term	Definition	Ref
CFU	A colony-forming unit is a unit used in microbiology to estimate the number of viable bacteria or fungal cells in a sample.	Link
CHARMM force field	A molecular simulation program with broad application to many-particle systems with a comprehensive set of energy functions, a variety of enhanced sampling methods**,** and support for multi-scale techniques including QM/MM, MM/CG, and a range of implicit solvent models.	Link
Cysteine	Cysteine is a nonessential amino acid (protein building block), meaning that cysteine can be made in the human body	Link
Dimer	A dimer is an oligomer consisting of two monomers joined by bonds that can be either strong or weak, covalent or intermolecular.	Link
MerA	MerA reduces Hg(II) to Hg(0) as a means of detoxification.	Link
MerB	MerB is a lyase that catalyzes the breaking of carbon-mercury bonds through protonolysis of toxic mercury compounds, such as methylmercury. This produces the less toxic and less mobile Hg^{2+} which is then completely volatilized to Hg^{0} by the enzyme MerA.	Link
MerC	MerC can transport Hg(II) into the cytosol	Link
MerE	MerE is a gene is part of the mer operon, a collection of bacterial genes specialized on the tolerance to various compounds of mercury including methylmercury.	Link
MerP	*MerP* codes for the enzyme. MerP is a periplasmic transporter that brings mercury into the cytoplasm of the cell.	Link

MerT	MerT is a 116 kDa transmembrane protein that assists in transporting Hg(II) into the cytoplasm of the bacterial cell.	Link
Methylmercury	Methylmercury is an organometallic cation with the formula $[CH_3Hg]^+$	Link
Michalis-Menten kinetics	The Michaelis-Menten equation has been used to predict the rate of product formation in enzymatic reactions for more than a century.	Link
ODE	In mathematics, an ordinary differential equation is a differential equation containing one or more functions of one independent variable and the derivatives of those functions	Link
PDB	The Protein Data Bank (PDB) archive is the single worldwide repository of information about the 3D structures of large biological molecules, including proteins and nucleic acids.	Link
Transcription	Transcription is the process by which the information in a strand of DNA is copied into a new molecule of messenger RNA (mRNA). DNA safely and stably stores genetic material in the nuclei of cells as a reference, or template.	Link
VMD	Visual Molecular Dynamics is a molecular modelling and visualization computer program	Link

5. References:

Brenda databse was used for kinetic values. https://www.brenda-enzymes.org/

Physiologyweb was used to create michaelis menton equation graph for the two enzymes. https://www.physiologyweb.com/calculators/michaelis_menten_equation_interactive_graph.html

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