Progress report

Search of potent inhibitors against target from *L. donovani* (Visceral Leishmaniasis) using Systems Biology, Virtual Screening and Molecular Dynamics

Objective 1: Literature survey and selection of the pathway

Metabolic pathways of the pathogens play an important role in the regulation of the pathogenicity and lethality. Therefore targeting essential pathways in the organisms and finding proteins playing an important role in the pathways could be beneficial.

Metabolic pathways of the pathogen *Leishmania donovani* expand the exploration space for potential drug targets against the protozoa. The KEGG database and extensive literature search enabled us to find the presence of around 98 different metabolic pathways for the organism *L. donovani*. However, only a few pathways are important for the pathogen survival and hence reported to be efficient for drug targeting. Some of the essential metabolic pathways of the pathogen are listed in Table 1.

Table 1: Important metabolic pathways

S No	Name of the pathway
1	Sterol Biosynthesis Pathway
2	Purine Salvage Pathway
3	Glycosylphosphatidylinositol (GPI) Pathway
4	Polyamine Biosynthesis Pathway
5	Trypanothione Synthesis Pathway
6	Sterol biosynthetic pathway
7	Glycolytic pathway
8	Redox metabolism pathway
9	Folate biosynthetic pathway
10	Mitogen-activated protein kinase (MAPK) pathway
11	Glutathione metabolism
12	Glyoxalase system

All the listed pathways have been thoroughly analysed. The pathways which has been already well studied and targets have been predicted, are not considered. Further, we need the

complete information about the pathways reactions, involved enzymes, products etc. and so the pathways with missing information have not been selected for next objective.

Two pathways which has been considered for the work are as follows –

a) **Glutathione metabolism**

The tripeptide thiol "glutathione" (l-γ-glutamyl- l-cysteinyl-glycine; GSH), plays a vital role in all living cells by performing crucial functions like transport, metabolism and cellular protection. The synthesis of glutathione proceeds in two steps, catalyzed by the enzymes γ-glutamylcysteine synthetase (glutamate-cysteine ligase) and GSH synthetase1 respectively. Another role of GSH is in the inactivation of drugs and processing of compounds like prostaglandins and oestrogens. Therefore inhibiting the thiol is one of the key mechanisms towards cellular inactivation.

Two enzymes namely trypanothione synthetase (TS) and trypanothione reductase (TR) is involved in the synthesis of trypanothione. Two molecules of glutathione and a molecule of spermidine are used for the catalysis process by TS. The intracellular maintenance of this dithiol is done through the action of an enzyme "trypanothione reductase", which is unique to this group of organisms. Being the only pathway involved in the regulation of the oxidative stress in these organisms, the trypanothione pathway has drawn attention for the development anti-trypanosomatid drugs.

b) **Purine Salvage Pathway**

The parasitic protozoa *Leishmania donovani* lacks the machinery for de novo synthesis of the purine nucleotides. The protozoan depends on their hosts for fulfilling their nucleotide requirement. Due to the reliability over an external source of purines, *Leishmania* have developed a specific pathway enabling them to transport the host purines into their nucleotide pool. Therefore they salvage the purine bases from the mammalian hosts through specific transporters located in the cell surface.

The nucleosides functions as an important regulator for most of the physiological processes and therefore regulation of the nucleoside transport is an important aspect of the parasitic protozoans. Most of the vital functions of the cells are carried out by the purines and the pyrimidines. Lacking the machinery for the de novo synthesis of purine nucleotides unlike their mammalian hosts, *Leishmania* has to rely on their host for their survival.

Objective 2: Biochemical network construction and modelling of selected pathway

a) Data Collection and filtration

Data retrieval and Protein interactions for the overall proteome of *L. donovani* have been retrieved from the Protein Interaction repository "STRING". A total of 437053 number of unique interactions were retrieved which consisted of 3707 proteins. The data was filtered to remove unwanted interactions and sort out the only relevant interactions. Out of the 3707 proteins, only 2175 proteins were curtained to be involved in around 27000 interactions.

b) Network of the selected pathways

The pathways that were essential for the survival of the pathogen were selected for our study. The main objective of pathways selection lies in the fact that proteins identified from these pathways will be involved in the survival of the pathway. A detailed literature survey for the pathways was carried out to find out the enzymes involved in the chemical reactions of the pathways. A total number of 18 enzymes have been found to be involved in the Glutathione metabolism pathway and 14 enzymes has been found to be involved in the Purine salvage pathway. The lists of enzymes are tabulated in Table 2 and Table 3.

Table 2: List of enzymes involved in the Glutathione metabolism

SI. No	Enzymes	EC number
1	5-oxoprolinase, putative	3.5.2.9
2	Gamma-glutamylcysteine synthetase	6.3.2.2
3	Leucyl aminopeptidase	3.4.11.1
4	Glutathione-specific gamma-glutamylcyclotransferase	4.3.2.7
5	Glutathione synthase	6.3.2.3
6	Isocitrate dehydrogenase	1.1.1.42
7	6-phosphogluconate dehydrogenase	1.1.1.44, 1.1.1.343
8	glucose-6-phosphate 1-dehydrogenase	1.1.1.49, 1.1.1.363
9	Spermidine synthase	2.5.1.16

10	Ornithine decarboxylase	4.1.1.17
11	Trypanothione synthetase/amidase	6.3.1.9, 3.5.1.-
12	Glutathionylspermidine synthase	6.3.1.8
13	Glutathione peroxidase	1.11.1.9
14	Trypanothione reductase	1.8.1.12
15	Ribonucleoside-diphosphate reductase subunits	1.17.4.1
16	Cytosolic tryparedoxin peroxidase,	1.11.1.15
17	Glutathione peroxidase-type tryparedoxin peroxidase	1.11.1.-
18	L-ascorbate peroxidase	1.11.1.11

Table 3: Enzymes involved in the purine salvage pathway

Sl. No	Enzymes	EC Number
1	Adenosine Kinase	2.7.1.20
2	Adenine Phosphoribosyltransferase	2.4.2.7
3	Adenine aminohydrolase	3.5.4.2
4	Purine Nucleoside Phosphorylase	3.2.2.1
5	Adenosine Deaminase	3.5.4.4
6	AMP deaminase	3.5.4.6
7	Adenylosuccinate Synthase / Succino-AMP synthetase	6.3.4.4
8	Adenylosuccinate lyase/ Succino-AMP lyase ADSL	4.3.2.2
9	Hypoxanthine-Guanine phosphoribosyltransferase	2.4.2.8
10	Xanthine Phosphoribosyltransferase	2.4.2.22
11	GMP Reductase	1.7.1.7
12	IMPDH	1.1.1.205
13	GMP Synthase	6.3.5.2
14	Guanine Deaminase	3.5.4.3

Several enzymes and metabolites constitute the pathways. The enzymes listed in the above tables interact with other proteins to perform their desired functions. The enzymes involved in the catalysis process were found to belong from several classes like lyases,

kinases, transferases etc. Therefore protein interactions for these tabulated enzymes were extracted out from the available data. We have summarized all the possible reactions and transformations and which will be used to construct a generalized form of the pathway. The constructed pathway should be able to maintain all the characteristics of the biological system and include all the components of the pathway involved in purine salvaging.

The conversion of the reactants is an important aspect for regulation of the pathway. These tabulated enzymes along with the enzymatic reactions that they catalyze are shown below (Table 4).

Table 4: Enzymatic reactions of the purine salvage pathway

Sl. No	Enzymes	Reactions
1	Adenosine Kinase	Adenosine + ATP -> adenosine 5' monophosphate + ADP
2	Adenine Phosphoribosyltransferase	AMP + diphosphate = adenine + 5-phospho-alpha-D-ribose 1-diphosphate
3	Adenine aminohydrolase	Adenine + H_2O → hypoxanthine + NH_3
4	Purine Nucleoside Phosphorylase	Purine nucleoside + phosphate ⇌ purine + alpha-D-ribose 1-phosphate
5	Adenosine Deaminase	Adenosine + H_2O → inosine + NH_3
6	AMP deaminase	AMP → IMP
7	Adenylosuccinate Synthase / Succino-AMP synthetase	GTP + IMP + L-aspartate = GDP + phosphate + adenylosuccinate
8	Adenylosuccinate lyase/ Succino-AMP lyase ADSL	Succino-AMP = AMP + fumarate
9	Hypoxanthine-Guanine phosphoribosyltransferase	1) IMP + Diphosphate = Hypoxanthine + 5-phospho-alpha-D-ribose 1-diphosphate 2) GMP + Diphosphate = Guaninee + 5-phospho-alpha-D-ribose 1-diphosphate
10	Xanthine Phosphoribosyltransferase	XMP + Diphosphate = 5-phospho-alpha-D-ribose 1-diphosphate + xanthine
11	GMP Reductase	GMP + NADPH + H^+ → IMP + NADP+ + NH_3
12	IMPDH	IMP + NAD+ + H_2O → XMP + NADH + H^+

13	GMP Synthase	ATP + XMP + NH_3 → AMP + diphosphate + GMP
14	Guanine Deaminase	Guanine + H_2O → xanthine + NH_3

The purine salvage pathway consisted of a total of 15 reactions with around 11 metabolites participating in it. Also, 14 enzymes were found to be involved in these reactions generating the desired metabolites. Table 5 is giving the enzymes and their enzymatic mechanism that has been obtained.

Table 5: Enzymes involved in Purine salvage pathway and their reaction mechanism

Enzymes	**Reaction mechanism**
Adenosine Kinase	Ordered Bi-Bi
Adenine Phosphoribosyltransferase	Ordered Bi-Bi
Adenine aminohydrolase	Henri-Michaelis-Menten analysis (irreversible)
Purine Nucleoside Phosphorylase	Ordered Bi-Bi
Adenosine Deaminase	Henri-Michaelis-Menten analysis (irreversible)
AMP deaminase	Henri-Michaelis-Menten analysis (irreversible)
Adenylosuccinate Synthase / Succino-AMP synthetase	Mass action (reversible)
Adenylosuccinate lyase/ Succino-AMP lyase ADSL	Ordered uni-bi ordered mechanism
Hypoxanthine-Guanine phosphoribosyltransferase	Ordered Bi-Bi
Xanthine Phosphoribosyltransferase	Ordered Bi-Bi
GMP Reductase	Bi (Irreversible)
IMPDH	Bi (Irreversible)
GMP Synthase	Mass action (reversible)
Guanine Deaminase	Henri-Michaelis-Menten analysis (irreversible)

Optimization of the constructed network using the above parameters is undergoing for the selected pathways. We will compare both the pathways after the formation if optimized networks.

Anupam Nath Jha

Publications (project acknowledged):

1) Shweta Jakhmola, Zaved Hazarika, **Anupam Nath Jha***, Hem Chandra Jha. In silico analysis of antiviral phytochemicals efficacy against Epstein–Barr virus glycoprotein H. Journal of Biomolecular Structure and Dynamics 2021 (https://doi.org/10.1080/07391102.2020.1871074)

2) Abhichandan Das, Upasana Pathak, Sanchaita Rajkhowa and **Anupam Nath Jha***. "Plasmodium falciparum: Experimental and Theoretical Approaches in Last 20 Years" in the edited book entitled Current Topics and Emerging Issues in Malaria Elimination (ISBN: 978-1-83968-484-5) (10.5772/intechopen.96529)

3) Sanchaita Rajkhowa, Zaved Hazarika and **Anupam Nath Jha***. "Systems biology and bioinformatics approaches in leishmaniasis" in the edited book entitled Applications of Nanobiotechnology for Neglected Tropical Diseases (ISBN: 978-0-12-821100-7) (https://doi.org/10.1016/B978-0-12-821100-7.00018-2)

4) Zaved Hazarika, Sanchaita Rajkhowa and **Anupam Nath Jha***. "Role of Force Fields in Protein Function Prediction" in the edited book entitled Homology Molecular Modeling - Perspectives and Applications (ISBN: 978-1-83962-806-1) (10.5772/intechopen.93901)

Anupam Nath Jha

UTILISATION CERTIFICATE

From 29/04/2019 to 17/06/2021

1. Title of the Project: Search of potent inhibitors against target from *L donovani* (Visceral Leishmaniasis) using Systems Biology, Virtual Screening and Molecular Dynamics

2. Project ID: 116

3. Principal Investigator and affiliation: Dr. Anupam Nath Jha, Assistant Professor, Tezpur University

4. Total amount sanctioned: Rs. 12,76,000.00 Date: 29-04-2019

5. Amount available for expenditure during the complete duration (2019-2021): Rs. 6,00,000.00

6. Amount brought forward from the previous financial year quoting SANCTION letter No. & date in which the authority to carry forward the said amount was given: Rs. Nil

7. Other receipts/interest earned, if any, on the grants: Rs 2070.00

8. Total amount that was available for complete duration: Rs 6,02,070.00

9. Actual expenditure: Rs. 5,97,743.00

10. Unspent balance refunded, if any (Please give details of cheque No. etc.): Rs. 4,327.00

11. Balance amount available at the end of the complete duration: Rs. 0.00

12. Amount allowed to be carried forward: Rs. 0.00

Certified that the amount of Rs. **5,97,743.00** mentioned against point no. 9 has been utilized on the project for the purpose for which it was sanctioned and that the balance of Rs. **4,327.00** was returned on project completion dated 17/06/2021.

Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled / are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Signature & Seal: Principal Investigator

Dr. Anupam Nath
Assistant Professor
Dept of Molecular Biology & Biotechnology
Tezpur University, Tezpur - 784028

20/9/2021

Signature & Seal: Finance Officer

Finance Officer
Tezpur University

22/9/21

Signature & Seal: Registrar/Head of Institution

Registrar
Tezpur University

For M. K. SEWDA & CO.
CHARTERED ACCOUNTANT
FRN- 330035E
M. Sewda
Mahendra Kr. Sewda
Proprietor
M. No.- 311963

*Note: Consolidated Statement of Accounts & Utilization Certificate,which is to be submitted on completion of the project. These forms shall be audited by Statutory Auditor (Government Auditor) or Chartered Accountant (external). However, the UC and SA audited by the internal auditor are accepted provided the accounts of the institution are audited by the C&AG and **same is certified by the Head of the Institution**. In respect of the other institutions where there is no audit by C&AG, they are required to submit the UC & SA audited by Statutory auditor/Chartered Accountant.

Statement of Expenditure

"Search of potent inhibitors against target from *L donovani* (Visceral Leishmaniasis) using Systems Biology, Virtual Screening and Molecular Dynamics"

Showing grants received from NECBH and the expenditure incurred during the period from **29/04/2019 to 17/06/2021**

(Rs. In Lakhs)

Item	Grants received from NECBH	Other receipts/ interest earned if any, on the grants	Total Amount (2+3)	Expenditure incurred during the complete duration	Balance (4-5)	Remark
1	2	3	4	5	6	7
A. Non –Recurring						
Equipment	2.75	Nil	2.75	2.74838	0.00162	
B. Recurring						
Manpower	3.00	Nil	3.00	2.75	0.25	
Consumables	0.00	Nil	0.00	0.00	0.00	
Travel	0.00	Nil	0.00	0.00	0.00	
Contingency	0.00	Nil	0.00	0.14602	- 0.14602	
Overheads (if applicable)	0.25	Nil	0.25	0.33303	- 0.08303	
Interest Earned	Nil	0.02070	6.02070	0.00	0.02070	
Total	**6.00**	**0.02070**	**6. 02070**	**5.97743**	**0.04327**	

Balance amount: Four Thousand Three Hundred and Twenty Seven only

20/9/21

Signature & Seal: Principal Investigator

Dr. Anupam Nath Jha
Assistant Professor
Dept of Molecular Biology & Biotechnology
Tezpur University, Tezpur - 784028

20/9/21

Signature & Seal: Finance Officer

Finance Officer
Tezpur University

20/9/21

Signature & Seal: Registrar/Head of Institution

Registrar
Tezpur University

For M. K. SEWDA & CO.
CHARTERED ACCOUNTANT
FRN- 330035E
M. Sewda.
Mahendra Kr. Sewda
Proprietor
M. No.- 311963

UDIN:- 21311963AAAAI3G289