The *Anopheles gambiae* Insecticidal Targets Made Bare by *In-silico* Analysis

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***Abstract*—several works had attempted to use genomics to explain the mode of mosquito resistance and predict drug target. The use of insecticides in various ways has been the major malaria vector control strategy being deployed lately, mostly pyrethroid, the major recommended compound class for IRS, ITNs and LLITNs. Resistance to drugs and insecticides has continually obstructed vector/malaria control strategies. The advert effect is so enormous in the Sub-Saharan African; its socioeconomic impact is unquantifiable in every measure. Thus, the quick necessity for the development and elucidation of potent, cheap and efficient new potential insecticidal targets, especially those in the class pyrethroid for the malaria vector, *A. gambiae*. In this work, an updated *Anopheles gambiae* biochemical metabolic network (AnoCyc ver1.0), otherwise known as pathway genome database (PGDB) was extracted, the database was reconstructed by developing a computational graph model in an approach that modeled the metabolic network of the organism as a bipartite graph, deployed the concept of choke point, load point and reaction without deviation to determine the essential enzymatic reactions in the networks. Each potential drug target to their corresponding gene/protein and such encoding protein sequences were extracted. (PDB) was blasted for genes that have structure or homologue of >= 30 sequence identity. Finally, we deployed Overton and Barton Score (OB-Score) and ParCrys prediction to rank proteins by their likely success in crystallization. 61 potential insecticidal candidate targets was made bare, one clinically validated insecticidal target and others with biological evidence in the literature. Seven of these targets ideally stand out and have no homology with other vertebrates. These in depth dissection of the biochemical metabolic networks of the Anopheles effectively identified the ideal gene products and specifically extract essential enzymes as new potential insecticidal target against A. gambiae.**

Index Terms*—*Anopheles gambiae, metabolism, insecticidal targets, in-silico, essential enzymes

I. INTRODUCTION

Insecticide resistance is an inherited characteristic involving changes in one or more insect genes [17, 18]. It is also a major public health challenge combating world efforts on malaria control. The malaria vector *Anopheles gambiae* (*A. gambiae*) has developed resistance to all existing classes of insecticides, particularly pyrethroids (the only class approved for Indoor Residual Spray [IRS] and Long-Lasting Insecticide Treated Net [LLITNs]) [1]. Identification of novel insecticidal targets for the development of more effective insecticides is a critical issue [2]. However, deciding which gene products are ideal insecticidal targets remains a difficult task. In the malaria parasite *Plasmodium falciparum*, Fatumo et al. [3,4] developed a computational method to investigate the topology of biochemical metabolic networks to mine new viable enzymatic drug targets which some were subsequently validated experimentally, [6]. Uniquely it was discovered that glutamyl-tRNA (gln) amidotransferase of *P. falciparum* can be inhibited by 6-diazo-5-oxonorleucine. This has been confirmed by an *in-vivo* study observing *P. berghei* infected mice [6]. We envisaged that the dissection and comprehensive study of biochemical metabolic networks has great potential to effectively and specifically identify essential enzymes as potential insecticidal targets against *A. gambiae*.

The completion of the genome sequence of the malaria vector, *A. gambiae* [7] has also given opportunity to develop various methods to facilitate effective malaria control strategies. Thus, in a previous work, we used the PathoLogic program [8] to construct AnoCyc ver1.0, a pathway/genome database (PGDB) for *A. gambiae* AgamP3, using its annotated genomic sequence and other annotated information from UNIPROT and KEGG databases. The resulting first PGDB for *A. gambiae* AgamP3 was deployed under the www.bioCyc.org databases (http://biocyc.org/ANO2/organism-summary?object=ANO2). This database was downloaded and rigorously analyzed in this study.

II. MATERIALS AND METHODS

A. Reconstructing the metabolic network

We extracted the metabolic network data of *A.gambiae* AgamP3 from AnoCyc database, http://www.biocyc.org, Version 18.1). The metabolic network of *A. gambiae* AgamP3 was modeled as a bipartite graph consisting of alternating nodes and edges as described previously [3,5,6]. For completion, in brief, this network was a connected graph which was established by defining neighbors of reactions: two reactions were neighbors if a metabolite existed that were the product of one reaction and the substrate for the other.

This yielded a bipartite graph of alternating reaction and metabolic compound nodes

B. *Computational analysis of the reconstructed metabolic network*

We used the concept of Choke Point (CP) to analyze the structure of our metabolic network [9,10]. A reaction is a chokepoint if it consumes a unique substrate and produces a unique product, making it indispensable. Inactivating choke-points could lead to an organism's failure. The number of true predictions out of all predictions of their approach is often large and sometimes difficult to prioritized [3,4], this makes it difficult for an experimentalist to choose the appropriate potential drug target when developing inhibitors as effective therapeutics [9].

As a combinatorial approach, we adapted the concept of Reaction without Deviation (RWD) [3,4] to further analyse our network. RWD are otherwise known as essential reactions. RWD analysis identified reactions within the network that are choke-points for which there are no other routes (deviations) within the network to replace the reactions. Figure 1 [3,4] indicates that an enzymatic reaction is considered a potential drug targets when such a reaction is ONLY a choke point (CP) as well as reaction without deviation (RWD). It also confirms that the knocked out reaction is a choke-point but may not be essential for the organism if the dashed lines exist in the metabolic network. Our approach inspects the network for such deviations. Thus, the RWD analysis investigates a reaction by deleting such a reaction from the metabolic network and further checks whether a chosen product could still be produced without the deleted reaction. This method can test multiple knock-outs merged with isolating essential enzymes on a high throughput manner to predict effective drug combinations [3,5].

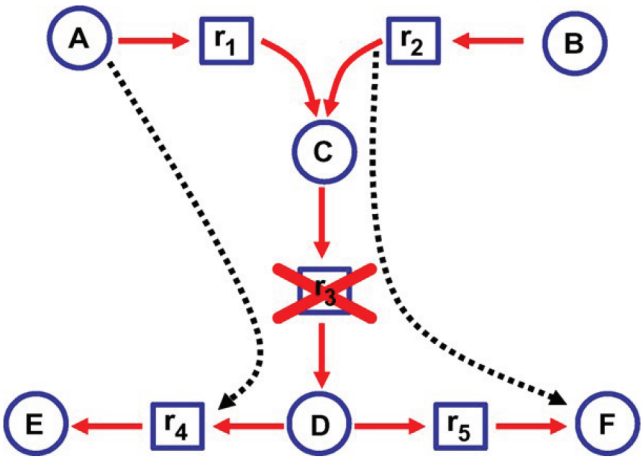

Fig. 1. The knocked out reaction is a choke-point but may not be essential for the organism if the dashed lines exist in the metabolic network. Our approach inspects the network for such deviations. (Reactions are boxes while metabolites are circles). [3,4].

III. RESULTS

Choke points analysis yielded 448 reactions, while implementing the RWD analysis yielded 645 reactions. 156 reactions were found to have their reversed copy included and were regarded as a duplicate of the forward reaction captured, therefore every duplicated reaction was discarded and we were left with 489 reactions. We compared the list of possible insecticidal target as captured by both the CP and RWD analysis, 264 reactions falls into the category of set of reactions that forms a tie (Interception) between the two methods (TABLE I.).

TABLE I. IMPORTANT STATISTICS FROM CP AND RWD ANALYSIS

Description	Quantity
Total number of CP enzyme before sieving for duplicates	896
CP Enzymes captured after sieving	448
Total number of RWD enzymes before sieving	645
RWD enzymes captured after sieving	489
Interception of CP + RWD = TIE	264

Each reaction on the tie (intercept) list of 264 reactions was matched to its corresponding enzymatic gene or protein. Targets that had more than one catalyzing enzymes and those without a valid corresponding enzymatic gene/protein and EC number were discarded, reducing the tie list to 173 enzymes. We extracted encoding protein sequences for each of the 173 enzymes from the Uniprot database server, where 153 out of 173 has their encoding sequences on this database. A total of 9 gene IDs were not resolvable. We again performed a comprehensive Position Specific Iteration alignment search on PDB database to determine if any of the protein sequences has a structure or homologue removal of 30% PDB sequence identity cut off. All potential targets with >30% identity to protein in PDB were discarded. In total 128 proteins on the target list had a <=30% identity to proteins in PDB. A PSI-BLASTp of every sequence against the Human, Bird (Chicken) and Fish (Tilapia) genome databases was performed to eliminate targets with homology to non-target organisms. Finally, OB & ParCrys [13,14], prediction was used to rank the 128 protein sequences according to their estimated likely success in crystallization. OB & ParCrys estimates a protein's propensity to produce diffraction quality crystals and its predictions are intended to provide guidance in selecting best targets (Table 2). The top 61 proteins on the predicted targets list classified as high scoring were selected as our final list of predicted insecticidal targets. We discarded the others.

TABLE II. OB & PARCRYS RATING TABLE FOR PREDICTED TARGETS LIST

OB & ParCrys Ranking	No of Genes in Class
High scoring	61
Amenable	33
Recalcitrant	31
Irresolvable	3
Total	128

TABLE 1. of the Appendix detailed the complete list of these 61 predicted insecticidal targets.

Next we compared these 61predicted enzymes with the gold standard list of 20 experimentally validated targets as represented on table 3 below. Those highlighted in red color are the enzymes that were not found on our reconstructed network. The rest of the enzymes were found on our network and 5 of them were captured as CP and / or RWD enzymes.

TABLE II. of the appendix summarizes the results of their homology tests with protein transcripts of the Human, Bird (Chicken) and Fish (Tilapia) genomes. Those highlighted in green have no significant homology (all E-values > 0.01) with Human and any other of the two organisms. See Tables 7, 8 and 9 of the supplementary material for full details of these homology tests.

A. The Gold Standard List

The gold standard list is a collection of proposed drug/ insecticidal targets from literature. On scanning a variety of established databases of insecticidal bank and literatures, we gathered a total of 20 reactions contained on our network. Of the 20 list, 11 were not found on our network. We extracted from various literature, 3 targets of clinically proven insecticides and the remaining 9 were proposed insecticidal targets, with proposal based on biological validation/ evidence via *in vitro* growth inhibition of the *A. gambiae*. To equally compare all predictions with the gold standard, every network on the network was mapped to its corresponding enzyme classification (EC) number. To measure the performance estimation, reactions without EC number were discarded. The complete list of the gold standard is given in TABLE III.

TABLE III. THE GOLD STANDARD LIST

EC Number	REACTION	References
1.1.1.145	3-beta –hydroxysteroid dehydrogenase/Delta 5--->4-isomerase	Curr Med Chem. 2008
1.4.3.4	Amine oxidase [flavin containing] B	Emerging drugs in neuropathic pain. Expert Opin Emerg Drugs. 2007
1.13.11.27	4-hydroxyphenylpyruvate dioxygenase	Expert Opin Emerg Drugs. 2009 Jun
1.14.15.6	Cytochrome P450 11A1, mitochondrial	Nucleic Acids Res. 2008 Jan
2.4.1.34	1,3-Beta-Glucan synthase	Mini Rev Med Chem. 2007
3.1.1.7	Acetylcholine esterase	J Med Chem. 2005 Feb 10
2.3.1.178	Diaminobutanoate acteyltransferase	Peters et al., 1990
2.6.1.46	Diaminobutyrate--pyruvate transaminase.	Vandenende et al., 2004
2.6.1.96	4-aminobutyrate—pyruvate transaminase	Van Cauwenberghe, O.R. and Shelp, B.J, 1999
1.3.7.1	6-hydroxynicotinate reductase.	**Alhapel et al., 2006**
1.17.1.5	Nicotinate dehydrogenase.	Yang et al., 2010
1.17.2.1	Nicotinate dehydrogenase	Yang et al., 2009
1.17.3.3	6-hydroxynicotinate dehydrogenase	**Wieser et al., 1997**
2.4.2.11	Nicotinate phosphoribosyltransferase	Hara et al., 2007
2.7.1.173	Nicotinate riboside kinase	Tempel et al., 2007, plos comp.
4.2.1.23	Spinosyn A	Tempel et al., 2007, plos comp
4.1.4.12	Tetraethylammonium-r(TEA)	Tempel et al., 2007, plos comp
4.1.14.7	Bungarotoxin (α7-nAChR)	Tempel et al., 2007, plos comp
1.1.11.141	Gamma-glutamyltransferase-rxn	San Framcisco inclusion, 2007
1.1.1.42	poly(oxy-1,2-ethanediyl),alphaisodecyl-omegahydroxy-phosphate	San Framcisco inclusion, 2007.

IV. CONCLUSION

Using these methods we computationally predicted list of 61 insecticidal targets for *A. gambiae*. This includes one with clinical validation. Out of these we identified 7 targets with no significant homology to Human, tilapia or chicken. For the known 20 experimental validated targets, out of the 9 found on our reconstructed network, 5 were detected by our computational analysis. Table 1 of the appendix elucidates the details of the 61 predicted insecticidal targets and information available for each of them from literature.

It is also clear from our analysis of the PGDB databases and data [19] that there is need for further rigorous pursuit of the manual and automatic curation of the biochemical metabolic network for *A. gambiae*.

Our analysis of the *P. falciparum* network has revealed that curation helped to close gaps and link up dangling ends. Thus, Following the results in our work on *P. falciparum* in [3,4,6], and on *A. gambiae* in [19], we derived insecticidal targets in *A. gambiae* with no significant homology to Human and also to Bird (Chicken) and Fish (Tilapia). These can be confirmed on the appendix tables and supplementary document/ data.

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APPENDIXES

TABLE IV. THE 61 PREDICTED INSECTICIDAL TARGETS AND INFORMATION AVAILABLE FOR EACH FROM LITERATURE

NAME	EC	GENE ID	Remarks	Reference	PDB ID
3-beta-hydroxy-delta5-steroid dehydrogenase	1.1.1.145	AGAP005984	May be involved in pheromones/hormones synthesis. **May be a good target.**	Simard J. et al. , 2005.	4DQV:A
3-hydroxyacyl-coa dehydrogenase	1.1.1.35	AGAP007784	Involved Fat metabolism, lipid degradation/metabolism (May not be a strong target)	Hillmer and Gottschalk, 1974	4B3H:A
Peroxid-rxn	1.11.1.7	AGAP000396	(A good target) involved in oxidation of toxic reductants, response to environmental stresses such as wounding, pathogen attack and oxidative stress	Rapoport et al., 1994	4G2E:A
Flavin-containing monooxygenase	1.14.13.8	AGAP010401	Detoxifying enzyme. Organisms have several mechanisms for detoxification. Its inhibition may not lead to death directly	Uno et al., 2013	4A9W:A
Cytochrome P450, family 3, subfamily A	1.14.14.1	AGAP012295	Very good target	J. G Scott, 1999	3S79:A
Cytochrome P450, family 3, subfamily A	1.14.14.1	AGAP012294	Very good target (Oxidizes xenobiotics and steroids, Participates in the metabolism of an as-yet-unknown biologically active molecule that is a participant in eye development)	K. G Scott, 1999	3V8D:A
Ecdysone-20-monooxygenase-rxn	1.14.99.22	AGAP002429	**Good target**. Involved in synthesis of hormone that controls molting. Insects generally depend on molting for growth. **NB: It may take longer time to kill the insect.**	Smith et al., 1979; 1983	3N9Y:A
Deoxyhypusine-monooxygenase-rxn	1.14.99.29	AGAP002129	A good target. Essential for organism's viability and plays a role in a wide number of important processes such as cell growth and proliferation regulates induction of autophagy and protein synthesis.	Ober & Hartmann, 1999	
Aldehyde dehydrogenase (NAD+)	1.2.1.3	AGAP003652	Involved in cellular metabolic process	Yoshida et al., 1998	4E3X:A
Aldehyde dehydrogenase (NAD+)	1.2.1.3	AGAP003578	Involved in cellular metabolic process	Yoshida et al., 1998	
Dihydlipoxn-rxn	1.8.1.4	AGAP011629	Good target. Involved in hyperactivation of spermatazoa during capacitation and so on.	Lamirande et al., 1993; http://enzyme.expasy.org/EC/1.8.1.4	4EQS:A
Hexaprenyldihydroxybenzoate methyltransferase	2.1.1.114	AGAP010537	Catalyzes the ubiquinone biosynthetic	Marbois et al., 1994	4HTF:A
Glycine N-methyltransferase	2.1.1.162	AGAP002198	Unknown function		3THR:A
23S rrna (uridine2552-2'-O)-methyltransferase	2.1.1.166	AGAP004177	Known for catalytic activity	http://www.chem.qmul.ac.uk/iubmb/enzyme/EC2/1/1/166.html	3DOU:A
18S rrna (adenine1779-N6/adenine1780-N6)-dimethyltransferase	2.1.1.183	AGAP004465	Involved in ribosome biogenesis.	Henras et al., 2008; http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/enzymes/GetPage.pl?ec_number=2.1.1.183	4ADV:A
2-octaprenyl-methoxy-benzoq-meth-rxn	2.1.1.201	AGAP010488	Ubiquinone biosynthesis	http://www.genome.jp/dbget-bin/www_bget?ec:2.1.1.201	3MGG:A

Trna (adenine57-N1/adenine58-N1)-methyltransfe rase	2.1.1.220	AGAP003966	tRNA processing (May be a slow target)	http://www.genome.jp/dbget-bin/www_bget?ec:2.1.1.220	2B25:A
Trna (guanine9-N1)-methyltransferase	2.1.1.221	AGAP000324	tRNA processing (May be a slow target)	http://www.genome.jp/dbget-bin/www_bget?ec:2.1.1.221	4FMW:A
Trna-guanine-n7--methyltransferase-rxn	2.1.1.33	AGAP004752	tRNA processing (May be a slow target)	http://www.genome.jp/dbget-bin/www_bget?ec:2.1.1.33	3DXY:A
Cap-specific mrna (nucleoside-2'-O-)-methyltra	2.1.1.57	AGAP000826	tRNA processing (May be a slow target)	http://www.genome.jp/dbget-bin/www_bget?ec:2.1.1.57	PDB:3IVD_A
Mrna (2'-O-methyladenosine-N6-)-methyltransfer ase	2.1.1.62	AGAP002895	tRNA processing (May be a slow target)	http://www.brenda-enzymes.org/php/result_flat.php4?ecno=2.1.1.62	PDB:2CDH_N
Protein-glutamine gamma-glutamyltransferase	2.3.2.13	AGAP009098	Involved in hemolymph clot. Not sure it will be a good target for speedy mortality.	http://www.genome.jp/dbget-bin/www_bget?ec:2.3.2.13	2XZZ:A
Gamma-glutamyltranspeptidase	2.3.2.2	AGAP008915	Involved in transferase activities	Meister, 1974; http://www.genome.jp/dbget-bin/www_bget?ec:2.3.2.2	3G9K:D
Farnesyl diphosphate synthase	2.5.1.1	AGAP007104	Good target. Catalyzes a precursor for several classes of essential metabolites	http://www.brenda-enzymes.info/php/result_flat.php4?ecno=2.5.1.1	PDB:2O1O_A
Gppsyn-rxn	2.5.1.1	AGAP004513	Good target. Catalyzes a precursor for several classes of essential metabolites	http://www.brenda-enzymes.info/php/result_flat.php4?ecno=2.5.1.1	PDB:2W48_A
Alkylglycerone-phosphate-synthase-rxn	2.5.1.26	AGAP004358	Lipid biosynthensis/metabolism	http://www.brenda-enzymes.org/php/result_flat.php4?ecno=2.5.1.26; Brown and Snyder, 1982.	PDB:3FW9_A
Ceramide-kinase-rxn	2.7.1.138	AGAP002932	Unknown (Coenzyme a biosynthensis)	http://www.brenda-enzymes.org/php/result_flat.php4?ecno=2.7.1.138	PDB:3VZB_A
Pantothenate-kin-rxn	2.7.1.33	AGAP010073	Unknown (Coenzyme a biosynthensis)	http://www.brenda-enzymes.org/php/result_flat.php4?ecno=2.7.1.33	PDB:2YXH_A
Pyramkin-rxn	2.7.1.35	AGAP005929	Unknown (Coenzyme a biosynthensis)	http://www.brenda-enzymes.org/php/result_flat.php4?ecno=2.7.1.35	PDB:2DDW_A
Ethanolamine-kinase-rxn	2.7.1.82	AGAP000010	(Coenzyme a biosynthensis)	http://www.brenda-enzymes.org/php/result_flat.php4?ecno=2.7.1.82	PDB:3C5I_A
Sphingosine kinase	2.7.1.91	AGAP006995	**Good target**. Involved in many cellular processes especially production of lipid secondary messengers.	http://www.brenda-enzymes.org/php/result_flat.php4?ecno=2.7.1.91	PDB:2JGR_A
DNA primase large subunit	2.7.7	AGAP006387	DNA replication and other complexes/ synthesis.	http://www.ebi.ac.uk/interpro/entry/IPR016558	PDB:1ZZ1_A

Gluconolact-rxn	3.1.1.17	AGAP010866	Y	http://www.ebi.ac.uk/intenz/query?cmd=SearchEC&ec=3.1.1.17	PDB:2J3E_A
3-hydroxyisobutyryl-coa hydrolase	3.1.2.4	AGAP004097	Involved in valine/protein catabolism	http://www.brenda-enzymes.org/php/result_flat.php4?ecno=3.1.2.4	PDB:4JYL_A
N-acylneuraminate-9-phosphatase-rxn	3.1.3.29	AGAP004391	Involved in a number of cellular activity and will be a good but slow target.	http://www.brenda-enzymes.org/php/result_flat.php4?ecno=3.1.3.29	PDB:3DDH_A
Polynucleotide-5-phosphatase-rxn	3.1.3.33	AGAP003517	Carbonhydrate metabolism, unsure of functions		PDB:3ICA_A
Multiple inositol-polyphosphate phosphatase	3.1.3.62	AGAP003555	Involved in feeding and digestion of blood-feeding insects	http://www.genome.jp/dbget-bin/www_bget?ec:3.1.3.62	PDB:1IHP_A
Inositol polyphosphate-4-phosphatase	3.1.3.66	AGAP000124	inositol phosphate metabolic process	http://www.genome.jp/dbget-bin/www_bget?ec:3.1.3.66	PDB:1UPQ_A
Ppgppsyn-rxn	3.1.7.2	AGAP004597	May be a good target. Enzyme involved in starvation response	www.berkeleybop.org/obo/MetaCyc:PPGPPSYN-RXN	PDB:2B9C_A
Beta-galactosidase	3.2.1.23	AGAP002058	Carbonhydrate metabolizer.	http://www.genome.jp/dbget-bin/www_bget?ec:3.2.1.23	PDB:3OG2_A
Betagalactosid-rxn	3.2.1.23	AGAP002055	Carbonhydrate metabolizer.	http://www.genome.jp/dbget-bin/www_bget?ec:3.2.1.23	PDB:1TG7_A
Beta-N-acetylhexosaminidase	3.2.1.52	AGAP005381	Carbonhydrate metabolizer.	http://www.genome.jp/dbget-bin/www_bget?ec:3.2.1.52; http://www.brenda-enzymes.info/php/result_flat.php4?ecno=3.2.1.52	PDB:3SUV_A
L-iduronidase	3.2.1.76	AGAP010549	May be a slow killer target.	Rome et al., 1978.	PDB:1PX8_A
Omega-amidase-rxn	3.5.1.3	AGAP012662	A very good target	http://www.brenda-enzymes.org/php/result_flat.php4?ecno=3.5.1.3; http://www.genome.jp/dbget-bin/www_bget?enzyme+3.5.1.3	PDB:3HKX_A
Peptide deformylase	3.5.1.88	AGAP003861	A very good target. Involved in protein biosynthensis	http://enzyme.expasy.org/EC/3.5.1.88	PDB:2AIA_A
Allantoicase-rxn	3.5.3.4	AGAP004501	Utilization of purines as secondary nitrogen sources, when primary sources are limiting	Probst et al., 2005	PDB:3OYT_A
Histidine-decarboxylase-rxn	4.1.1.22	AGAP009001	Catecholamine biosynthesis	Entrez Gene: histidine decarboxylase	PDB:2OKK_A
Dent gamma-carboxylase	4.1.1.90	AGAP006363	A good target (involved in blood clotting)	http://www.ebi.ac.uk/intenz/query?cmd=SearchEC&ec=4.1.1.90	PDB:1RTS_A
Deoxyribose-p-ald-rxn	4.1.2.4	AGAP009161	Unknown function		PDB:3OA3_A

Myo-inositol-1-phosphate-synthase-rxn	5.5.1.4	AGAP011830	Lipid and Inositol biosynthe Lipid and Phospholipid metabol and Phospholipid biosynthesis.	Donahue & Henry, 1981; Majumder et al., 1997	PDB:3QVW_A
Serine--trna-ligase-rxn	6.1.1.11	AGAP008265	**Good target**. Participates in Translation of mRNA to protein. Blocking it will inhibit several biochemical processes especially neuropeptide hormones	Berg et al., 1961	PDB:1WLE_A
Aspartate--trna-ligase-rxn	6.1.1.12	AGAP007844	**Good target**. Participates in Translation of mRNA to protein. Blocking it will inhibit several biochemical processes especially neuropeptide hormones	Allen et al., 1960	PDB:4DPG_A
Proline--trna-ligase-rxn	6.1.1.15	AGAP003589	**Good target**. Participates in Translation of mRNA to protein. Blocking it will inhibit several biochemical processes especially neuropeptide hormones	Bergmann et al., 1961	PDB:3UH0_A
Cysteine--trna-ligase-rxn	6.1.1.16	AGAP011821	**Good target**. Participates in Translation of mRNA to protein. Blocking it will inhibit several biochemical processes especially neuropeptide hormones	Berg et al., 1961; Allen et al., 1960	PDB:2X1M_A
Phenylalanine--trna-ligase-rxn	6.1.1.20	AGAP003517	**Good target**. Participates in Translation of mRNA to protein. Blocking it will inhibit several biochemical processes especially neuropeptide hormones	Berg et al., 1961; Allen et al., 1960	PDB:2CXI_A
Histidine--trna-ligase-rxn	6.1.1.21	AGAP000735	**Good target**. Participates in Translation of mRNA to protein. Blocking it will inhibit several biochemical processes especially neuropeptide hormones	Berg et al., 1961; Allen et al., 1960	PDB:3OD1_A
Leucine--trna-ligase-rxn	6.1.1.4	AGAP008297	**Good target**. Participates in Translation of mRNA to protein. Blocking it will inhibit several biochemical processes especially neuropeptide hormones	Berg et al., 1961; Allen et al., 1960	PDB:1WZ2_A
Isoleucine--trna-ligase-rxn	6.1.1.5	AGAP002101	**Good target**. Participates in Translation of mRNA to protein. Blocking it will inhibit several biochemical processes especially neuropeptide hormones	Berg et al., 1961; Allen et al., 1960	PDB:1QU3_A
Long-chain-fatty-acid--coa ligase ACSBG	6.2.1.3	AGAP008596	Involved in lipid and fatty acid metabolism	Berg et al., 1961; Allen et al., 1960	PDB:3PBK_A
Ubiquitin--protein-ligase-rxn	6.3.2.19	AGAP005577	Involved majorly in cell growth and differentiation. Will affect larvae more.	Ardley and Robinson, 2005; Dou et al., 2012.	PDB:2CNX_A
Magnesium chelatase	6.6.1.1	AGAP004956	Very good pesticide target. Involved in bacteriochlorophyll pigment biosynthesis	Al-Karadaghi, 2001; Walker and Weinstein, 1991	PDB:1LTL_A