Covenant University, Ota

CODE MALARIA:
ERADICATION DEVELOPMENTS FOR THE DECADE

Ezekiel Adebiyi

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Synopsis

The malaria (parasite) is believed to have its origin in the West and Central Africa. The tropics provide ideal breeding and living conditions for the malaria vector, *Anopheles* mosquito. The parasites spread to other areas through the journey of man, following the human migrations to the Mediterranean, Mesopotamia, the Indian peninsula and South-East Asia. And by 19th century, malaria reached its global limits with over one-half of the world's population at significant risk and 1 in 10 affected expected to die from it. From most part of Europe and north America, 1955 - 1969, malaria was eradicated using diligently a combination of, for example, Chloroquine and DDT with provision of infrastructures that allow application of DDT to breeding areas without undue exposure of DDT to man.

Reports have shown that the resistance of the parasite to existing drugs is increasing. Therefore, there is a huge and urgent need to discover and validate new drug or vaccine targets to enable the development of new treatments for malaria. Every year 300 million to 500 million people suffer from this disease (90% of them in sub-Saharan Africa). About 1.5 million to 3 million people die of malaria every year (85% of these occur in Africa). One child dies of malaria somewhere in Africa every 20 sec., and there is one malarial death every 12 sec somewhere in the world. Malaria kills in 1 year what AIDS killed in 15 years. If required infrastructures improve dramatically in Saharan Africa, incidence of malaria will reduce by 80% (B. M. Greenwood, Personal Comm., 2009).

A number of solutions for the treatment of malaria are in development. Principal among them is the late-stage trials malaria vaccine, RTS or Mosquirix for children, driven by the Pharmaceutical giant, GSK, but its effectiveness declines slightly over time. A semi-synthetic version of the antimalarial drug artemisinin developed (development started Dec., 2004) by UC Berkeley's Jay Keasling is moving out of development into full-scale production by sanofi-aventis Pharmaceutical. This has been made possible by a $53.3 million grant from the Bill & Melinda Gates Foundation. The drug, produced by genetically engineered bacteria, is much cheaper than the plant-derived drug available today. It is yet unknown how resistance of the parasite will play out for this drug as resistance of the parasite to the plant derived one exist and biological mode of actions of the drug is unknown. At Covenant University with collaborators from Universities of Heidelberg and Marburg, Germany, using novel techniques from bioinformatics, we have computation-

ally mined four (4) enzymatic drug target sites on the malaria parasite for which the biological mode of actions of associated bioactive compounds will be entirely known. This discovery provides for the first time antimalarial drug target sites upon which a viable structural design pipeline is being built. And also provides a viable platform to optimize the fitting of "indigenous" medicinal plants bioactive compounds via a rational drugs design approach. We have also recently discovered other drug target sites, such as signaling pathways and transcription factors and are developing techniques towards diagnosing malaria at the liver stage. In collaboration from John Hopkins University, USA and Imperial College, UK with about $800k initial grant (under review) from Bill & Melinda Gates Foundation, we are developing an evolution-proof insecticide, equipped with the effective capability of Dichlorodiphenyltrichloroethane (DDT) but designed to target only malaria infected mosquitoes. Lastly, in collaboration from University of Heidelberg, Germany, MRTC, Mali and the John Hopkins University, USA, we are also developing models for successful deployment of Sterile Insect Technique (SIT) also towards the eradication of malaria infected mosquitoes.

Acknowledgements

My first acknowledgement goes to God, whose hand upon my life has made the execution of the work presented in this lecture possible. The uniqueness of His hand upon my life can be summarized using 1 Kings 18, 46: **"And the hand of the LORD was on Elijah; and he out ran the chariot of Ahab to the entrance of Jezreel."**

I appreciate the Chancellor of Covenant University, Dr David Oyedepo, a number of his quotes have inspired me and are still inspiring me. One of such reads: **"Every problem in a place has a solution provider for that problem there."**

I also appreciate our amiable Vice Chancellor, Prof Aize Obayan, who has released herself to be used of God in moving the University forward; for her unrelenting pursuit of excellence.

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2. Dr Benedikt Brors, Division of Theoretical Bioinformatics, German Cancer Research Center (DKFZ), Heidelberg, Germany;
3. Dr Marcus Oswald, Institute of Computer Science, University of Heidelberg, Heidelberg, Germany.
4. Prof Roland Eils, Division of Theoretical Bioinformatics, German Cancer Research Center (DKFZ), Heidelberg, Germany and BIOQUANT, University of Heidelberg, 69120 Heidelberg, Germany;
5. Prof Micheal Lanzer, Department of Parasitology, University of Heidelberg, Germany;
6. Prof Ginsburg Hagai, Department of Biochemistry, Hebrews University of Jerusalem, Isreal;
7. Prof Seydou Doumbia, Malaria Research and Training Center (MRTC), University of Bamako, Mali;
8. Prof Micheal Schlitzer, Department of Chemistry, University of Marburg, Marburg, Germany;
9. Prof Nancy Amato, Department of Computer Science, Texas A&M, University, College Station, USA;
10. Dr Lars Kaderali, BIOQUANT, University of Heidelberg, Heidelberg, Germany;
11. Dr George Christophides, Division of Cell and Molecular Biology, Imperial College, London;
12. Prof Fotis Kafatos, Division of Cell and Molecular Biology, Imperial College, London;
13. Dr Jason Rasgon, JHMRI, Johns Hopkins University, Baltimore, USA;
14. Prof Marcelo Jacobs-Lorena, JHMRI, Johns Hopkins University, Baltimore, USA; and
15. Dr Manuel Llinas, Dept. of Molecular Biology, Princeton University, USA.

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research cluster. These also includes persons from the Biological Sciences department that are working with me in malaria related projects. They are worthy labourers in this vineyard.

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5. Fulbright

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Contents

Introduction

1.1 The malaria disease, origin and early history

Malaria is probably the only infection that can be treated in just three days, yet that kills millions every year. Without prompt and appropriate treatment, malaria may become a medical emergency by rapidly progressing to complications and death[66]. The term malaria is derived from the Italian 'mal'aria', which means 'bad air', from the early association of the disease with marshy areas[94].

Malaria[94] is transmitted through the bite of an infected female *Anopheles* mosquito. Of the approximately 400 species of *Anopheles* throughout the world, about 60 are malaria vectors under natural conditions, 30 of which are of major importance. Malaria parasites are *eukaryotic* single-celled microorganisms that belong to the genus *Plasmodium*. More than 100 species of *Plasmodium* can infect numerous animal species such as reptiles, birds and various mammals, but only four species of parasite can infect humans under natural conditions: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. These four species differ morphologically, immunologically, in their geographical distribution, in their relapse patterns and in their drug responses. *P. falciparum* is the agent of severe, potentially fatal malaria and is the principal cause of malaria deaths in young children in Africa[92]. The least common malaria parasite is *P. ovale*, which is restricted to West Africa, while *P. malariae* is found worldwide, but also with relatively low frequency. The most widespread malaria parasite is *P. vivax*

but infections with this species are rarely fatal. Although *P. falciparum* and *P. vivax* can both cause severe blood loss (anemia), mild anemia is more common in *P. vivax* infections, whereas severe anemia in *P. falciparum* malaria is a major killer in Africa. In addition, in the case of *P. falciparum*, the infected erythrocytes can obstruct small blood vessels and if this occurs in the brain, cerebral malaria results, a complication that is often fatal, particularly in African infants. *P. ovale* and *P. vivax* have dormant liver stages named hypnozoites that may remain in this organ for weeks to many years before the onset of a new round of pre-erythrocytic schizogony, resulting in relapses of malaria infection. In some cases *P. malariae* can produce long-lasting blood-stage infections, which, if left untreated, can persist asymptomatically in the human host for periods extending into several decades.

Carter and Mendis[81] hypothesized that the end of the last glacial period and warmer global climate heralded the beginnings of agriculture about 10000 years ago. It is argued that the entry of agricultural practice into Africa was pivotal to the subsequent evolution and history of human malaria. The Neolithic agrarian revolution, which is believed to have begun about 8,000 years ago in the "Fertile Crescent," southern Turkey and northeastern Iraq, reached the western and Central Africa around 4,000 to 5,000 years ago. This led to the adaptations in the *Anopheles* vectors of human malaria. The human populations in sub-Saharan Africa changed from a low-density and mobile hunting and gathering life-style to communal living in settlements cleared in the tropical forest. This new, man-made environment led to an increase in the numbers and densities of humans on the one hand and generated numerous small water collections close to the human habitations on the other. This led to an increase in the mosquito population and the mosquitoes in turn had large, stable, and accessible sources of blood in the human population, leading to very high anthropophily and great efficiency of the vectors of African malaria. Even though the practice of agriculture had developed throughout the tropics and subtropics of Asia and the Middle East up to several thousand years before those in Africa, simultaneous animal domestication in Asia probably prevented the mosquitoes from developing exclusive anthropophilic habits. In most parts of the world, the anthropophilic index (the probability of a blood meal being on a human) of the vectors of malaria is much less than 50% and often less than 10 to 20%, but in sub-Saharan Africa, it is 80 to almost 100%. This is probably the most important single factor responsible for the stability and intensity of malaria transmission in tropical Africa today.

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Thus by 19th century, malaria reached its global limits with over one-half of the world's population at significant risk and 1 in 10 affected expected to die from it. From the time of the voyages of Columbus until the mid-19th century, European trade and colonization in the tropics were marked by enormous losses of life from malaria. On the coasts of West Africa, mortality rates often exceeding 50% of a company per year of contact were the norm. From the mid-19th century onward, with the use of the Cinchona bark, mortality rates fell rapidly to less than one-quarter of this. Up to early 20th century, repeated untreated infections of *P. vivax* and prolonged infections of *P. malariae* also contributed significantly to the mortality along with the most lethal *P. falciparum*. **Poor living conditions, poverty and famine probably contributed to the high mortality.** During the past 100 years, nearly 150 million to 300 million people would have died from the effects of malaria, accounting for 2-5% of all deaths. In the early part of the century, malaria probably accounted for 10% of global deaths and in India

it probably accounted for over half. Countries popularised the need to eradicate this menace, via advertisements that included stamps with mosquito. Some of these stamps are shown below:

Figure 1.1: Some countries stamps with mosquito picture. Source: http://www.cdc.gov/malaria/about/history/

Towards the end of the 19th century, Charles Louis Alphonse Laveran, a French army surgeon, noticed parasites in the blood of a patient suffering from malaria, and Dr Ronald Ross, a British medical officer in Hyderabad, India, discovered that mosquitoes transmitted malaria and "explained that malaria did not emanate from the marshes as was believed, but from pots and tubs thrown everywhere". The Italian professor Giovanni Battista Grassi subsequently showed that human malaria could only be transmitted by *Anopheles* mosquitoes.

The life cycle of malaria parasites is extremely complex and requires specialized protein expression for survival in both the invertebrate (mosquito) and vertebrate (e.g., human) hosts. These proteins are particularly required for both intracellular and extracellular survival, for the invasion of a variety of cell types and the evasion of host immune responses . Using the most lethal malaria parasite, *P. falciparum*, the full life cycle of malaria can be depicted as found in Figure 1.2.

All the clinical symptoms and pathological changes seen during human infection are caused by the asexual blood stages (otherwise known as the Intraerythrocytic Developmental Cycle (IDC)) of Plasmodium. The IDC is the target for the vast majority of antimalarial drugs and vaccine strategies. Within host red blood cells (RBCs), the parasite undergoes enormous developmental changes during its maturation. The first round of IDC takes 48 hours. Observe that this cycle, if not broken can be infinite, leading to

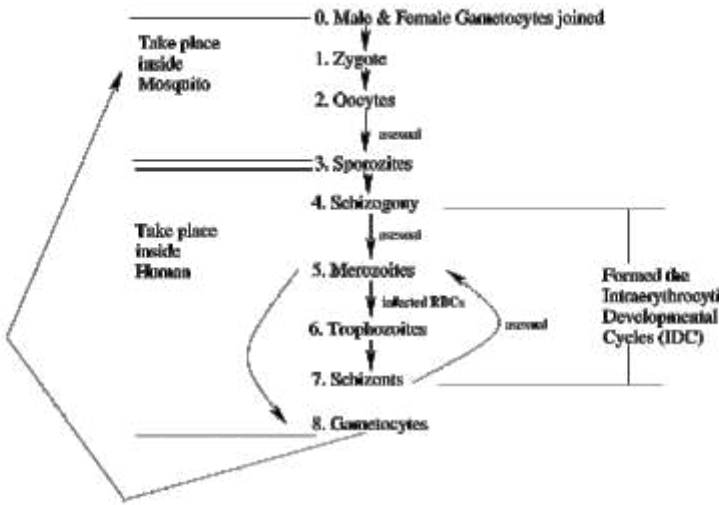

Figure 1.2: Life cycle of the *Plasmodium falciparum* in Man(a) and Mosquito(b).[4]

the break-down of the system of the host. Using Bozdech *et al.*[19], this can describe as follows. IDC initiates with merozoite invasion of RBCs and is followed by the formation of the parasitophorous vacuole (PV) during the ring stage. The parasite then enters a highly metabolic maturation phase, the trophozoite stage, prior to parasite replication. In the schizont stage, the cell prepares for reinvasion of new RBCs by replicating and dividing to form up to 32 new merozoites. In some more detail(Ben Mamoun *et al.*[17]), the ring stage takes the first 20-24h (after invasion), and beginning from 20-24h (after invasion), develops to a trophozoite stage. The trophozoite stage then undergoes multiple rounds of DNA replication and nuclear divisions, giving rise to schizont-stage parasites (beginning $\approx$ 36h after invasion). The final stage, called the late schizont or segmenter stage (42-48h after invasion), is characterized by the production of individual merozoites within the infected erythrocyte. Note that each of these merozoites is capable of infecting a new erythrocyte. The genetic machinery that controls this developmental process has not yet been identified. Also some of these merozoites can instead form into gametocytes by turning on or off certain genes. These gametocytes can then be picked up by feeding mosquitoes.

Below in Figures 1.3 and 1.4 are two other figures that encapsulate this life cycle, more graphically.

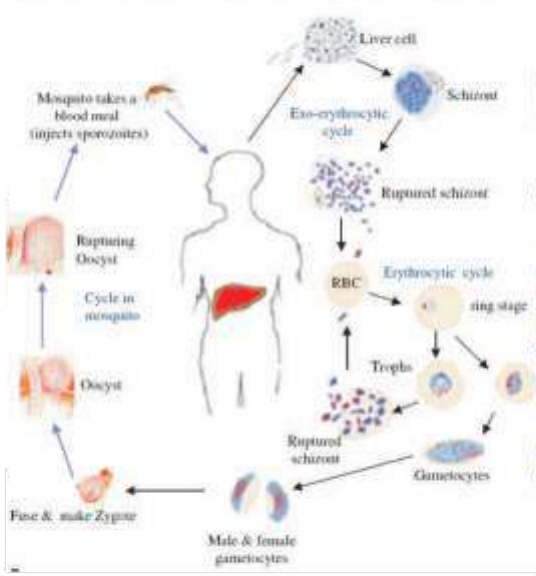

Figure 1.3: Life cycle of the parasite *P. falciparum*. The figure has been prepared with the help of the information, artwork and micrographs from the CDC's websites for parasite identification http://www.dpd.cdc.gov/dpdx and http://www.itg.be.[94]

1.2 Symptoms and Diagnosis

The accumulation and sequestration of parasite infected RBCs in various organs such as the heart, brain, lung, kidney, subcutaneous tissues and placenta is a characteristic feature of infection with *P. falciparum*. Sequestration is a result of the interaction between parasite-derived proteins, which are present on the surface of infected RBCs, and a number of host molecules expressed on the surface of uninfected RBCs, endothelial cells and in some cases placental cells[14].

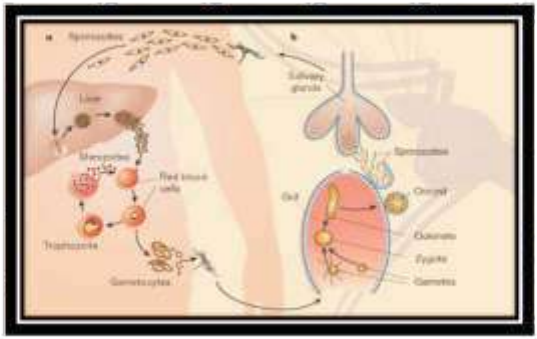

Figure 1.4: Life cycle of the *Plasmodium falciparum* in Man(a) and Mosquito(b).[103]

Malaria symptoms can develop as soon as 6-8 days after being bitten by an infected mosquito, or as late as several months after departure from a malarious area. People infected with malaria parasites typically experience fever, shivering, cough, respiratory distress, pain in the joints, headache, watery diarrhea, vomiting and convulsions[68]. Severe malaria is usually complex and several key pathogenic processes such as jaundice, kidney failure and severe anemia can combine to cause serious and often fatal disease[68]. Malaria is especially dangerous to pregnant women and small children and in endemic countries it is an important determinant of prenatal mortality[107]. Parasite sequestration in the placenta is a key feature of infection by *P. falciparum* during pregnancy and is associated with severe adverse outcomes for both mother and baby, such as premature delivery, low birthweight and increased mortality in the newborn[108]. After repeated exposure to malaria during pregnancy, women in areas of endemicity slowly develop immunity; thus multigravid women are comparatively less susceptible to pregnancy-associated malaria than primagravid women.

Malaria is diagnosed using a combination of clinical observations, case history and diagnostic tests, principally microscopic examination of blood[110]. Ideally, blood should be collected when the patient s temperature is rising, as that is when the greatest number of parasites is likely to be found. Thick blood films are used in routine diagnosis and as few as one parasite per 200μL blood can be detected. Rapid diagnostic 'dipstick' tests, which fa-

cilitate the detection of malaria antigens in a nger-prick of blood in a few minutes are easy to perform and do not require trained personnel or a special equipment[110]. However, they are relatively expensive and although *P. falciparum* can be diagnosed, *P. ovale*, *P. malariae* and *P. vivax* cannot be distinguished from one another using this method. Three consecutive days of tests that do not indicate the presence of the parasite can rule out malaria.

1.3 Treatments and their development stages

Malaria can be cure, control and prevent. Presently, there are various antimalarial drugs for curing the disease (from the blood stream of a malaria infected person) with special preference to one or the other based on location (see Fig 3.2 for example).

For controlling this menace, insecticides and bed nets are the available means, while prevention can be brought to bearing via the combine use of insecticides and antimalarial drugs. By the 1950s, this combination is what led to the elimination of malaria from the North America, Europe and Australia[120]. Another cheap mean is to use vaccine, but till date, there is no licensed vaccines[121].

To come by, with any of these treatments, the following stages must be followed painstakingly.

- Discovery
  - can take over 2 years (typically ongoing)
  - or watch the competition ("Me Too!" drugs)
- Preclinical testing
  - Lab and animal testing
- Phase 1
  - Limited number of healthy volunteers are given the drug
  - Safety and dosage determination
- Phase 2
  - 100 to 300 patient volunteers

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  - Lab and animal testing
- Phase 1
  - Limited number of healthy volunteers are given the drug
  - Safety and dosage determination
- Phase 2
  - 100 to 300 patient volunteers

  - Testing for efficacy and side effects
- Phase 3
  - 1000 to 5000 patient volunteers
  - Testing for long term reactions
- Regulatory governmental agencies review and approval
- Post-marketing testing

Time range to complete the above cycle is between 7 to 15 years.

Antimalarial efforts historically and short comings

2.1 Antimalarial drugs

In this section, we run through history, including ancient details about the development of antimalarial compounds dating back from the use of bark from plants.

The herb Artemisia annua (sweet wormwood) was known to the Chinese as qing-hao for more than 2000 years. The Mawanhgolui Han dynasty tombs, dating to 168 BC, mentioned it as a treatment for hemorrhoids. In 340 AD, the anti-fever properties of qinghao were first described by Ge Hong of the East Yin Dynasty. An appeal for help from Ho Chi Minh to Zhou En Lai during the Vietnam War triggered the work on this herb and in 1967, the Chinese scientists set up Project 523. The active ingredient of qinghao was isolated by Chinese scientists in 1971. An ethyl ether extract of qinghao fed to mice infected with the rodent malaria strain, Plasmodium berghei, was found to be as effective as chloroquine and quinine at clearing the parasite. The human trails were published in the Chinese Medical Journal in 1979. Many active derivatives of artemisinin have since been synthesized and it is today a very potent and effective antimalarial drug, particularly against drug resistant malaria in many areas of southeast Asia. So far, clinically relevant genetic resistance to artemisinin has not been reported, although tolerance has been noted.

The history of cinchona bark or later named quinine, of more than 350 years, is full of intrigue and drama, greatly influencing that of pharmacy, botany, medicine, trade, theoretical and practical chemistry and tropical agriculture. The origin of cinchona remains shrouded in mystery.

One of the tales attributes the identification of cinchona bark to South American Indians. These natives supposedly noted that sick mountain lions chewed on the bark of certain trees. Malaria patients were given the bark and were helped.

Another holds that a member of a Peruvian Spanish garrison first discovered the bark. This soldier, overcome by malaria, was left behind to die by his comrades. Tortured by thirst, he crawled to a shallow pond, where he drank deeply and fell asleep. On awakening he found that his fever had disappeared, and then he remembered that the water had a bitter taste. A large tree trunk, split by lightning, had fallen into the pool; the bark from this tree, the soldier soon discovered, had both the bitter taste and the remarkable power to cure malaria.

It is not very clear as to who brought the cinchona bark to Europe. Sebastiano Bado, an Italian, gives this honor to the Countess of Chinchon, in an account published in 1663. The fourth Count of Chinchon, Don Luis Geronimo Fernandez de Cabrera de Bobadilla Cerday Mendoza, was appointed by Philip IV to rule the vast Spanish South American Empire. The count and his wife, Senora Ana de Osorio, arrived in Lima in 1629. Shortly thereafter, according to Bado, the countess became severely ill with tertian fever, and news of her suffering soon spread throughout the colony. The governor of Loxa wrote the count, recommending that some of the same medicine by which he had been recently cured be given to Senora Ana. Don Juan was summoned to Lima, the remedy given, and the countess cured. Soon the natives were swarming around the palace, both to express their joy at the recovery and to learn the secret of the remedy. Upon hearing the people's pleas, the generous Senora Ana ordered a large quantity of the bark and gave it personally to the sick. The grateful sufferers, all of whom were cured, named the new remedy los polvos de la condea, "the countess' powder." In 1639, according to Bado, the countess returned to Spain, bringing a large quantity of bark with her. She distributed her remedy among the peons on the Chinchn estate, and also sent some to an ailing theology

professor at the University of Alcal de Henares. At the same time, Juan de Vega, Seora Ana's physician, who had also returned to Spain with a supply of bark, sold part in Seville at an exorbitant price, one hundred reals per pound. This unscrupulous practice was to be repeated by many men in many places before the precious bark became readily available.

The first partially successful separation of the active principle from cinchona was achieved in 1811 by a Portuguese naval surgeon named Bernadino A. Gomez. He extracted the gray bark of poor variety with dilute acid and then neutralized it with alkali and managed to obtain a few crystals which he named cinchonin (later, to be known as cinchonine).

French pharmacists, Joseph Pelletier and Joseph Bienaime Caventou, appointed a full professor of toxicology at the Ecole de Pharmacie in Paris at age 22, isolated a medicinally worthless quinine poor powder, from the gray bark in 1817. In 1819, Friedlieb Runge isolated a base from cinchona, which he named "China base" - which was different from cinchonine. Later, in 1820, Pelletier and Caventou isolated from the yellow bark a sticky, pale yellow gum that could not be induced to crystallize. The gum was soluble in acid, alcohol, and ether and highly effective against malaria. The properties of the gum were seen to be identical to "China" base; but Runge's prior discovery was overlooked. The two men named the new chemical quinine after quinquina, the name given by Peruvian Indians to the bark, meaning medicine of medicines or bark of barks. Pelletier and Caventou refused any profit from their discovery. Instead of patenting the extraction process, they published all the details so that anyone could manufacture quinine. They received many honors, the most lucrative of which was the Prix Monthyon of ten thousand francs awarded by the French Institute of Science. A monument was erected in Paris commemorating this achievement of Pelletier and Caventou.

Even today quinine remains an important and effective treatment for malaria in most parts of the world, although resistance has been reported sporadically in 1844 and 1910.

Many drugs were developed to protect the troops from malaria, particularly during World War II. Chloroquine, Primaquine, Proguanil, amodi-

aquine and Sulfadoxine/Pyrimethamine were all developed during this time.

During World War I, Java and its valuable quinine stores fell into Japanese forces. As a result, the German troops in East Africa suffered heavy casualties from malaria. In a bid to have their own antimalarial drugs, the German government initiated research into quinine substitutes and entrusted it to Bayer Dye Works. Most of the work was done at Bayer Farbenindustrie A.G. laboratories in Eberfeld, Germany. Several thousands of compounds were tested and some were found to be useful. Plasmochin naphthoate (Pamaquine) in 1926 and quinacrine, mepacrine (Atabrine) in 1932 were the first to be found. Plasmochin, an 8 amino quinoline, was quickly abandoned due to toxicity, although its close structural analog primaquine is now used to treat latent liver parasites of P. vivax and P. ovale. Atabrine, although found superior and persisting in the blood for at least a week, had to be abandoned due to side effects like yellowing of the skin and psychotic reactions. The breakthrough came in 1934 with the synthesis of Resochin (chloroquine) by Hans Andersag, followed by Sontochin or Sontoquine (3 methyl chloroquine). These compounds belonged to a new class of antimalarials known as 4 amino quinolines. But Farben scientists overestimated the compounds toxicity and failed to explore them further. Moreover, they passed the formula for Resochin to Winthrop Stearns, Farben's U.S. sister company, in the late 1930s. Resochin was then forgotten until the outbreak of World War II.

With the German invasion of Holland and the Japanese occupation of Java, the Allied forces were cut off from quinine. This stimulated a renewed search for other antimalarials both in the United Kingdom and in the United States. After the Allied occupation of North Africa, the French soldiers raided a supply of German manufactured Sontochin in Tunis and handed it over to the Americans. Winthrop researchers made slight adjustments to the captured drug and this new formulation was called chloroquine. Later, it was found to be identical to the older and supposedly toxic Resochin. However it was not available for the troops until the end of the War. But following World War II, chloroquine and DDT became the two principal weapons in the global malaria control campaign. However, after only about ten to twelve years of use, chloroquine resistance appeared in *P. falciparum*. Two initial foci of

resistance developed simultaneously in Colombia and on the Cambodia-Thailand border. From these loci, resistance spread throughout South America and southern Asia. By the late 1970s chloroquine resistance had reached Africa and has since spread across sub-Saharan Africa.

Other antimalaria drugs: The formula of Atabrine (mepacrine, a 9-aminoacridine), was also soon solved by Allied chemists and it was produced in large scale in the U.S. It immediately gained widespread acceptance as an excellent therapeutic agent. After the experiments of Brigadier N. Hamilton Fairley in Australia in 1943, it was also found to be useful as a prophylactic agent, protecting the troops in malarious areas. It is no longer used in view of many undesirable side effects. The success of chloroquine led to the exploration of many (nearly 15000) compounds in the United States and another 4-aminoquinoline Camoquin (amodiaquin) was discovered. Studies on 8-aminoquinolines led to the discovery of Primaquine by Elderfield in 1950. Meanwhile, British investigators at ICI also carried out extensive studies on malaria drugs and Curd, Davey and Rose synthesised antifolate drugs proguanil or Paludrine (chlorguanide hydrochloride) in 1944 and Daraprim or Malocide (pyrimethamine) was developed in 1952. However, resistance to proguanil was observed within a year of introduction in Malaya in 1947. *P. falciparum* strains resistant to pyrimethamine, and cross-resistant to proguanil emerged in 1953 in Muheza, Tanzania. Sulfadoxine-pyrimethamine combination was introduced in Thailand in 1967. Resistance to this was first reported in Thailand later that year and spread quickly throughout Southeast Asia and recently appeared in Africa.

Mefloquine was jointly developed by the U.S. Army Medical Research and Development Command, the World Health Organization (WHO/TDR), and Hoffman-La Roche, Inc. After World War II, about 120 compounds were produced at the Walter Reed Army Institute of Research and WR142490 (mefloquine), a 4-quinoline methanol was developed. Its efficacy in preventing and treating resistant *P. falciparum* was proved in 1974-75 and was useful for the US Army in Southeast Asia and South America. By the time the drug became widely available in 1985, evidence of resistance to mefloquine also began to appear in Asia.

In 1998 a new drug combination was released in Australia called Malarone. This is a combination of proguanil and atovaquone. Atovaquone be-

came available 1992 and was used with success for the treatment of Pneumocystis carrinii. The synergistic combination with proguanil is found to be an effective antimalarial treatment.

It is important to note that the plant-derived drugs have outlived many of the synthetic drugs, to which resistance has developed.

2.2 Insecticides and bed nets

As early as 1825 Michael Faraday reported to the Royal Society of London the formation of benzene hexachloride. However, it had to wait for more than 115 years to become useful as a pesticide. The use of chemicals to control troublesome insects so as to save food crops started by mid 19th century. Paris green was used as an insecticide in 1867. Production of pyrethrum, which is a natural insecticide derived from the chrysanthemum flower, started in the US by 1870. In 1882, Petroleum was first recommended in the US for insect bites and stings. By 1897 oil of citronella was used as insect repellent. Pyrethrum was first used by William Gorgas in Cuba where it was burned inside sealed dwellings. In around 1910, the German scientist G. Giemsa was experimenting with different ways of using pyrethrum and developed a way of spraying pyrethrum on walls with a spray pump. This method took over two decades to catch on, and it was used with great success in South Africa for the control of malaria on sugar estates. In 1920 Oil-soaked sawdust was first recommended for mosquito control and Paris green was considered as a mosquito larvicide. Paris Green was first used in malaria control in the 1920s. It was used in countries like India, South Africa and Brazil.

In 1924, Paris green dust was applied to swamps in Louisiana for control of *Anopheles* mosquitoes. In 1942, many chemicals were tested for control of insect-borne disease among Armed Forces. By 1947, more than 13,000 such chemicals had been tested and classified, but the glory went to Dichlorodiphenyltrichloroet -hane (DDT), resynthesised by Paul Muller in 1939; In 1943, Van Linden gave the name lindane to the pesticide made with the active isomers of the benzene hexachloride mixture.

DDT was first synthesized in 1874 by a Viennese pharmacist, Othmar Zeidler, he did not investigate the properties of the new substance but simply published his synthesis. Then in 1939 in Switzerland, Paul Mueller of the Geigy Company, resynthesized this compound and discovered its insecticidal

properties. The Geigy Company began to market the substance in 1940-41 as a 5% dust called Gesarol spray insecticide and a 3% dust called Neocid dust insecticide. The now universally used name, DDT, was first applied by the British Ministry of Supply in 1943. DDT was first added to U.S. Army supply lists in May 1943. Gahan and colleagues, in August 1943, made the first practical tests of DDT as a residual insecticide against adult vector mosquitoes. The first field test in which residual DDT was applied to the interior surfaces of all habitations and outbuildings of a community to test its effect on *Anopheles* vectors and malaria incidence was in Italy in the spring of 1944. This experiment was carried out in the town of Castel Volturno at the mouth of the Volturno River, north of Naples, by the Malaria Control Demonstration Unit of the Malaria Control Branch of the Public Health Sub-Commission, Allied Control Commission, Italy. Spraying began on 17 May 1944, and this experiment, together with a second one started later in the Tiber Delta area, lasted 2 years. The war needs and experiments greatly accelerated its acceptance and use and led to the discovery and application of similar insecticides such as benzene hexachloride and dieldrin.

Use of mosquito nets has been dated to prehistroic times. It is said that Cleopatra, Queen of Egypt, also slept under a mosquito net.

Eradication efforts worldwide

It should be said that the first intervention against malaria, that led to the ancient Roman drainage program, was borne out of the association of the disease with stagnant water (breeding grounds for *Anopheles*). It is said that Emperor Nero drained the swamps near ancient Rome, in order to rid the city of malaria.

3.1 First eradication campaign and setback: 1955-1978

Many drugs were developed to protect the troops from malaria, particularly during World War II. Chloroquine, Primaquine, Proguanil, amodiaquine and Sulfadoxine/Pyrimethamine were all developed during this time.

Following World War II, with the success of DDT, the advent of less toxic, more effective synthetic antimalarials like Chloroquine (embedable in household salt), and the enthusiastic and urgent belief that time and money were of the essence, the World Health Organization (WHO) submitted at the World Health Assembly in 1955 an ambitious proposal for the eradication of malaria worldwide[58, 43, 24]. Eradication efforts began and focused on

- house spraying with residual insecticides,
- antimalarial drug treatment, and
- surveillance,

and would be carried out in 4 successive steps:

1. preparation,
2. attack,
3. consolidation, and
4. maintenance.

Successes include elimination in nations with temperate climates and seasonal malaria transmission. Some countries such as India and Sri Lanka had sharp reductions in the number of cases, followed by increases to substantial levels after efforts ceased. Other nations had negligible progress (such as Indonesia, Afghanistan, Haiti, and Nicaragua). Some nations were excluded completely from the eradication campaign (most of sub-Saharan Africa). Sub-Saharan Africa was not included (or even ignored) due to its massive reservoir of malaria and insufficient infrastructure to support the programme. The emergence of drug resistance, widespread resistance to available insecticides, wars and massive population movements, difficulties in obtaining sustained funding from donor countries, and lack of community participation made the long-term maintenance of the effort untenable. However, by 1949 mosquitoes resistant to DDT and other new insecticides were found. In 1962, Rachel Carson published Silent Spring. In it, she discussed the decline in certain regions of the United States of the America robin, due to its consumption of earthworms that were laden with the DDT used in massive amounts to combat Dutch elm disease. Carson's book stimulated widespread public concern about DDT and other pesticides. Through a series of legal hearings in the United States instigated by lawyers and scientists working with the Environmental Defense Fund, DDT was eventually banned or severely restricted in most states. In 1972, the U.S. Environmental Protection Agency banned all DDT uses except those essential to public health. Similar bans were instituted by Sweden in 1969 and later in most of the developed countries.

Completion of the eradication campaign was eventually abandoned. But DDT is still being used in some developing countries to control malaria, but the debate is continuing. This studies have shown that Dichlorodiphenyltrichloroethane (DDT) is a powerful organo-chlorine pesticide against insect vectors of diseases such as malaria and typhus. No other chemical product is as effective and affordable as the DDT in repelling mosquitoes from homes, exterminating or disorientating insects that are not killed or repelled and

largely eliminating their urge to bite in homes that have been treated once or twice a year with tiny amounts. Due to its effectiveness and cost-wise advantage at killing insects, DDT, before being banned, has been a mainstay to fight malaria world-wide. DDTs careful and intelligent usage has helped recently Jamaica to be malaria free. In another studies in favour of the ban of DDT, it was found that prenatal exposure to DDT causes developmental delays. A recent study also showed that in-utero exposure to the organochlorine pesticide dichlorodiphenyltrichloroethane and its breakdown product, dichlorodiphenyltrichloroethane, indicate that exposure is associated with poorer infant (6months and older) and child neurodevelopment[31, 109]. Another study showed that polybrominated diphenylethers (PBDEs) and polychlorinated biphenyls (PCBs) can interact and enhance developmental neurobehavioral defects when the exposure occurs during a critical stage of neonatal brain development[80]. So the above dangers that can result from the usage of DDT with a potential of altering the destiny of the upcoming generations of the nations where it is persistently used without proper infrastructure is another motion in the continuing support of the ban of DDT.

For now, the goal of most current National Malaria Prevention and Control Programs and most malaria activities conducted in endemic countries is to reduce the number of malaria-related cases and deaths. To reduce malaria transmission to a level where it is no longer a public health problem is the goal of what is called malaria "control."

3.2 Present malaria situation

The graphical distribution of malaria world-wide is shown in the Figure below:

And the distribution with respect to, where chloroquine is no more effective is shown in Figure 3.2 below.

Malaria affects more than 2400 million people representing over 40% of the world's population, in more than 100 countries in the tropics from South America to the Indian peninsula. The tropics provide ideal breeding and living conditions for the *Anopheles* mosquito, and hence this distribution.

Every year 300 million to 500 million people suffer from this disease (90% of them in sub-Saharan Africa, two thirds of the remaining cases occur in six countries- India, Brazil, Sri Lanka, Vietnam, Colombia and Solomon Islands). WHO forecasts a 16% growth in malaria cases annually. About 1.5

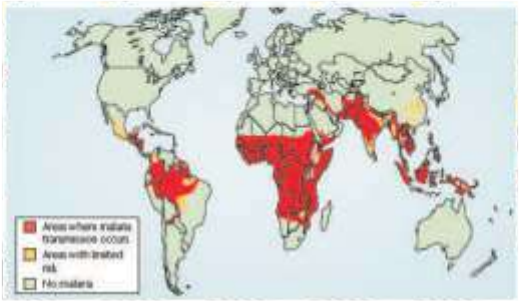


Figure 3.1: Distribution of malaria world-wide.[96]

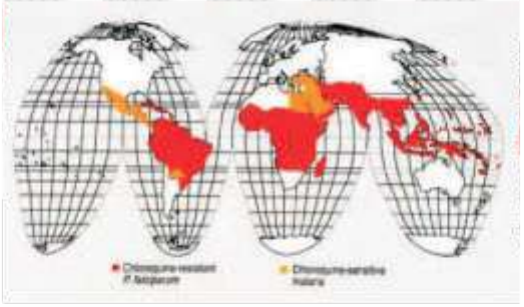


Figure 3.2: Distribution of malaria world-wide with locations where Chloroquine is no more effective.[66]

million to 3 million people die of malaria every year (85% of these occur in Africa), accounting for about 4-5% of all fatalities in the world. This can be graphically explained as crashing sixteen (16) air-buses loaded with 500 people on a daily basis of the year. One child dies of malaria somewhere in Africa every 20 sec., and there is one malarial death every 12 sec somewhere in the world. Malaria kills in 1 year what AIDS killed in 15 years. In 15 years, if 5 million have died of AIDS, 50 million have died of malaria.

Malaria ranks third among the major infectious diseases in causing deaths-after pneumococcal acute respiratory infections and tuberculosis. It is expected that by the turn of the century malaria would be the number one infectious killer disease in the world. It accounts for 2.6 percent of the total disease burden of the world and it is responsible for the loss of more than 35 million disability-adjusted life-years each year. Every year 30000 visitors to endemic areas develop malaria and 1% of them may die. The estimated global annual cost (in 1995) for malaria is US$ 2 billion (direct and indirect costs, including loss of labour). And the estimated worldwide expenditure on malaria research is US$ 58 million, which is one thousandth of the US$ 56 billion spent globally on health research annually. A total of US $ 84 million is the estimated annual expenditure on malaria research, prevention and treatment. A glemming scenario of how inadequate the malaria treatment development initiative has not been presently well funded is shown in the following figures. The estimated worldwide expenditure per malaria fatality is $ 65; as compared to $ 3274 for HIV/AIDS and $ 789 for asthma. That is to say, one HIV/AIDS death is equal to about 50 malaria deaths!

Early and effective chemotherapy for malaria has a pivotal role in reducing morbidity and mortality especially since a vaccine is unlikely to emerge within the next decade. Multidrug resistance has been reported from most parts of the world and as a result, monotherapy or some of the available combination chemotherapies for malaria are either ineffective or less effective. New antimalarial regimens are, therefore, urgently needed and antimalarial combination chemotherapy is widely advocated. Antimalarial combinations can increase efficacy, shorten duration of treatment (and hence increase compliance), and decrease the risk of resistant parasites arising through mutation during therapy. Combination therapy with antimalarial drugs is the simultaneous use of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite. The concept of combination therapy is based on the synergistic or additive potential of two or more drugs, to improve therapeutic efficacy and also delay the

development of resistance to the individual components of the combination. Artemisinin based combinations are known to improve cure rates, reduce the development of resistance and they might decrease transmission of drug-resistant parasites. The total effect of artemisinin combinations (which can be simultaneous or sequential) is to reduce the chance of parasite recrudescence, reduce the within-patient selection pressure, and prevent transmission. In Zambia, for example, convincing evidence of the failing efficacy of chloroquine resulted in the initiation of a process that eventually led to the development and implementation of a new national drug policy based on artemisinin-based combinational therapy (ACT)[91].

Antimalarial efforts (elsewhere): state or under development

Recent increases in resources, political will, and commitment have led again to discussion of the possibility of malaria treatment and control and, ultimately, eradication. This new dispenciation has been arguebly pursued rigoriously by Bill and Melinda Gates Foundation.

A number of solutions for the treatment of malaria are in development. Principal among them is the late-stage trials malaria vaccine, RTS or Mosquirix for children, driven by the Pharmaceutical giant, GSK, but its effectiveness declines slightly over time. A semi-synthetic version of the antimalarial drug artemisinin developed (development started Dec., 2004) by UC Berkeley's Jay Keasling is moving out of development into full-scale production by sanofi-aventis Pharmaceutical. This has been made possible by a $53.3 million grant from the Bill & Melinda Gates Foundation. The drug, produced by genetically engineered bacteria, is much cheaper than the plant-derived drug available today. It is yet unknown how resistance of the parasite will play out for this drug as resistance of the parasite to the plant derived one exist and biological mode of actions of the drug is unknown.

In the sections that follow, we will briefly give an enumeration of other initiatives.

4.1 Antimalarial drugs

We provide an overview of antimalarial drugs development elsewhere. This overview is taken from the Osamor Ph.D thesis[78] and Winzeler perspectives in [113].

Drugs are chemicals or other substances that alter the function of an organism and are referred as medicines or therapeutic drugs when used for the prevention, treatment and alleviation of diseases as opposed to other hard drugs, such as opiates, which are used illegally. Drugs can be derived from plant, mineral, animal, or synthetic sources. Many early folk medicines, including aspirin, opium, and quinine were derived from plants. Minerals used as medicines include boric acid, Epsom salts, and iodine. Many hormones used to treat a bodily malfunction include insulin for diabetes, or growth hormone to promote proper human development. The table below shows the list of some available malaria drugs as they evolve with time and fail due to resistance, non-compliance, safety and formulation issues[160].

Drug	Reg.(Yr)	Organisations
Mefloquine	1984	Hoffman La Roche, WRAIR
Halofantrine	1988	GSK, WRAIR
Artemether	1997	Malariaone Poulenc Rorer, Kunmig/TDR
Artemether-lumefantrine	1999	Novartis
Atovaquone+proguanil	2000	GSK
Artemotil (beta-arteether)	2000	Artecef, WRAIR / TDR
Chlorproguanil-dapsone	2003	GSK / TDR
Artesunate-Amodiaquine	2007	Sanofi-Aventis/DNDi

Table 4.1: Some Available Malaria Drugs Showing Evolution with Time. The table also shows respective organizations involved in various antimalarial drug development. GSK, GlaxoSmithKline; WRAIR, Walter Reed Army Institute of Research; WHO/TDR, World Health Organization Tropical Dieseases Research.(Source: Nwaka, 2008[160])

Natural products are the sources of the two most important drugs currently available to treat severe *P. falciparum* malaria, quinine and derivatives of artemisinin. In the case of artemisinin, relatively simple chemical modifications of the natural product parent compound have led to a series of highly potent antimalarials that are playing an increasingly important role in the

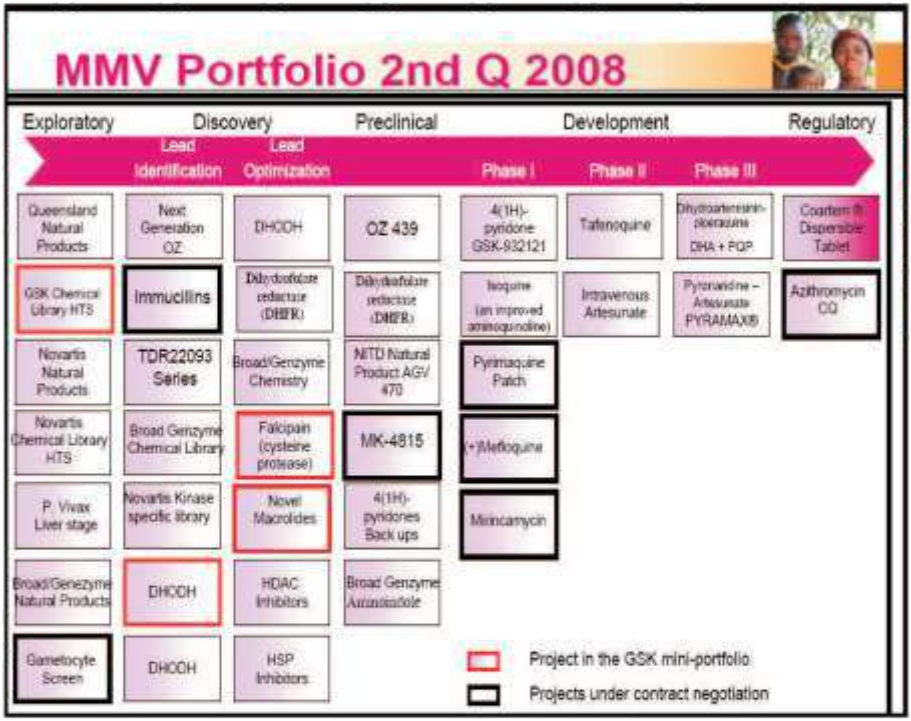
treatment of malaria[161]. However, the cost of these compounds may be limiting, and so efforts to design fully synthetic endoperoxides that are less expensive to produce are an important priority[162, 163].

Artesunate has been studied in combination with both sulfadoxine/pyrimeth and amodiaquine[165] in Africa, with good efficacy. Rosenthal[166] stated that artemisinin analogs, in particular artesunate and artemether, have recently shown great promise as rapidly acting as potent antimalarials, but the short half-lives of these compounds lead to many late recrudescences after therapy, as seen with artesunate/sulfadoxine/pyrimethamine in Uganda[167] suggesting that combination therapies are necessary to fully exploit the potency of this class.

Ideally, a combination regimen that prevents resistance development should include at least two agents against which parasite resistance has not yet developed and which have similar pharmacokinetics, so that low blood levels of a single agent will not be present. No such ideal regimen is currently available, although chlorproguanil/dapsone/artesunate may prove to fit this description. Alternatively, the combination of a short-acting, highly potent compound and a longer-acting agent may prove effective, if the initial decrease in parasite burden is so great as to limit subsequent resistance development to the long-acting agent (e.g. artesunate/mefloquine). As another alternative, two drugs with similar pharmacokinetics may prove effective even if resistance to each agent is present in the community (e.g. amodiaquine/sulfadoxine/pyrimethamine). Relatively slow-acting antimalarials (e.g. antibiotics) in combinations like quinine and doxycycline may be effective[166].

Initiatives like Medicine for Malaria Venture (MMV) had projects with drugs at various stages of development as at second quarter of 2008 (shown in Figure 4.1 below). From figure 4.1, we have the cherry taste and powdery form of Coartem dispersible (from Norvatis), newly formulated for children now at regulatory stage and waiting to be recognized for usage come late 2009. Also Azithromycin CQ is formulated to be safer antimalarial at pregnancy. They all pass from exploratory to regulatory stages drug development pipeline.

It is important to note that among drugs at various stage in MMV are enzymatic pathways found not in humans. The approaches used to find these sites are based on genome analysis, therefore, similar to our approaches. The MMV is also at the same stage we are at our antimalarial drugs discovery program: discovery of novel inhibitors to interrupt some of these pathways, such as the non-mevalonate pathway of isoprenoid biosynthesis[168].



(Source: Bathurst, 2008)

Figure 4.1: Medicine for Malaria Venture (MMV) Initiative Drug Discovery Portfolio: Drug projects at various stages of development.

4.2 Insecticides and bed nets

Indoor residual spraying (IRS) with insecticides and the insecticide-treated bed nets (ITNs) are mainstay tools for malaria control. IRS with DDT have been responsible for the elimination of malaria from many countries[95]. And more recently, ITNs have become a leading tool for malaria control[111] and Pyrethroid insecticides are the preferred choices[86, 63, 98]. DDT was banned due to its damaging effects (in particular, when used in areas with open guters, which are preminent features in areas, where presently the malaria challenge is more severe) on non-targets organisms, like humans, birds, etc[26, 31, 109, 80, 45, 51]. Apart from this, as in the last century, one of the major challenges to either IRS or the ITNs is the emergence of evolution of insecticide resistance in *Anopheles* populations[98, 63, 102, 86].

Presently, insecticides recommended for malaria control by the World Health Organization (WHO) represent just four classes of compound for IRS and just one class of compounds for ITNs[71, 25]. To manage resistance, a number of strategies have been developed, but resistance management requires on-going surveillance and a level of application management that is frequently problematic in regions where the malaria challenge is most prevelant[60, 22]. Therefore, there is a new renewed effort to identify new insecticidal compounds for use in malaria control[46, 119].

4.3 Vaccination

Vaccination has been the most cost-effective health intervention for a range of infectious diseases, and this should one day include malaria. An overview on the state of antimalarial vaccines can be found in [120, 121].

Most licensed vaccines generate antibodies against extracellular pathogens, which can be accurately measured and often correlate with protection. Such vaccines comprise whole inactivated microorganisms or, increasingly, parts (or subunits) of microorganisms with appropriate adjuvants. As early as 1967, experimental immunization with irradiated sporozoites was shown to generate protective immunity [12]. Proof-of-concept demonstrates that protection can indeed be induced through vaccination with a subunit vaccine was recently obtained in children [13,14]. From [129], we noted that protective immunity can be generated by immunization with irradiated sporozoites, by allowing infected and irradiated mosquitoes to take blood meals on hu-

man volunteers. T cells against liver-stage antigens are considered the main immune effectors in this case. However, the precise nature of the immune responses (effector mechanisms, antigen specificity and magnitude) that directly reduce or prevent malaria are unknown, and are often poorly modelled in animals[120]. This makes the search for a malaria vaccine all the more difficult. Furthermore, despite the *P. falciparum* genome sequence being known and many stage-specific antigens having been identified, there are differences in opinion over the appropriate antigen to be incorporated into a vaccine, and indeed over whether a vaccine should include multiple antigens and whether these should be from different stages of the lifecycle. However, antigens are usually assessed, initially at least, one at a time.

Several lines of evidence suggest that a prophylactic malaria vaccine for humans is feasible. Firstly, immunization of naive human volunteers with irradiated (and thus attenuated) sporozoites was shown to confer 90% sterile protection against experimental infection following laboratory-bred, sporozoite-infected mosquito bites [36,37]. Secondly, naturally acquired immunity progressively builds up during the first two decades of life in people living in malaria-endemic countries. This immunity primarily impacts the severity of clinical disease, and appears to be linked to continuous antigenic stimulation,waning rapidly when exposure ceases [10,38,39]. Thirdly, protection has been elicited by passive transfer of hyperimmune immunoglobulins from malaria-immune adults into malaria-naive human volunteers [11].

However, progress in developing a malaria vaccine has, however, remained slow [15]. This is in part due to the fact that **the *Plasmodium* parasite has more than 5200 genes** that could code for a protective antigen [16,17], making identification of candidate vaccine antigens a real quagmire, which is rendered even more complex by the fact that these antigens are differentially expressed during the life-cycle of the parasite and that many of the antigens display a high degree of variability. Moreover, the same antigens can be developed into different types of vaccines in a wide variety of ways. Thus, more than 50% of the approximately 75 candidate vaccines in active development today, are based on just three antigens that were cloned 20 years ago [18]: the circumsporozoite protein (CSP), the merozoite surface protein (MSP) and the apical membrane antigen 1 (AMA1) [19 21].

The traditional approach to develop malaria vaccines has focused on the targeting of one of the different stages of parasite development, whether the pre-erythrocytic, the asexual (intra-erythrocytic, blood stages) or the sexual stage. Pre-erythrocytic vaccine strategies aim to generate an antibody

response able to neutralize sporozoites and prevent them from invading the hepatocyte, as well as to elicit a cell-mediated immune response able to interfere with the intra-hepatic multiplication cycle of the parasites, e.g. by killing the parasite-infected hepatocytes. This type of vaccine would be ideal for travelers because it would prevent the advent of any form of clinical disease. Asexual blood-stage (erythrocytic stage) vaccine strategies aim to elicit antibodies that will inactivate merozoites and/or target malarial antigens expressed on the RBC surface through antibody-dependent cellular cytotoxicity and/or complement lysis; and also are meant to elicit T-cell responses able to inhibit the development of the parasite in RBCs. By decreasing the exponential multiplication of merozoites, this type of vaccine would mostly serve as a disease-reduction vaccine in endemic countries [40]. As for vaccines that target the sexual stage of the parasite, they do not aim to prevent illness or infection in the vaccinated individual, but to prevent or decrease transmission of the parasite to new hosts. This transmission-blocking vaccine can be seen as a true altruistic vaccine. Besides these classical approaches, novel approaches being currently undertaken include the development of an irradiated sporozoite vaccine [41] and an anti-parasite toxin vaccine that targets the parasite toxins which contribute to the disease, such as the glycosylphosphatidylinositol (GPI) anchor. Recent promising results obtained with knock-out sporozoites in mice will likely lead to further developments over the coming years [21,42,43].

From the foregone, vaccines under development (at highly advanced stages) can be categorized into three, namely pre-erythrocytic, asexual blood-stage (erythrocytic) and multistage. Three others include the 'transmission-blocking', an irradiated sporozoite and an anti-parasite toxin vaccine respectively. A table showing the first set and the stages they are (in development) is shown below:

The major malaria vaccine funding agencies are

- the NIH in the USA,
- the Wellcome Trust in the U.K.,
- the European Union, either directly through the European and Developing Countries Clinical Trials Partnership (EDCTP) or through the European Malaria Vaccine Initiative (EMVI),
- USAID,

Stage	Main target	Vaccine	Antigen	Phase	Location	Developer (Refs)
Pre-erythrocytic	Sporozoites	RTS,S–AS01/AS02	CSP	Ib/IIb	Multiple African sites	GSK[14]
	Hepatocytes	FP9/MVA, ME-TRAP	ME-TRAP	IIb	Kenya	University of Oxford[8,9]
	Hepatocytes	Simian adenovirus/MVA	ME-TRAP	Ia/Ib	UK	University of Oxford
	Hepatocytes	LSA-1–AS02	LSA-1	Ia/IIa	USA	WRAIR[15]
	Hepatocytes	AdHu35	CSP	Ia	USA	Crucell[16]
Blood stage	Merozoites	FMP1–AS02	MSP-1_{42}	Ib/IIb	Kenya/Mali	WRAIR[18]
	Merozoites	AMA1–AS02	AMA1	Ia/IIa	USA	WRAIR[17]
	Merozoites	MSP1_{42}–Alum, bi-allelic	MSP-1	Ia	USA	NIH[19]
	Merozoites	AMA1–FVO$_{(25-545)}$	AMA1	Ia	Netherlands	BPRC
	Merozoites	GMZ2	GLURP/MSP-3	Ia	Germany	SSI[18]
	Merozoites	PRCP2.9	AMA1/MSP-1_{19}	Ia	China	Wansing[20]
Multi-stage	Sporozoites, hepatocytes, merozoites	FP9/MVA polyprotein	Six antigens	IIa	UK	University of Oxford[9]
	Sporozoites, merozoites	PEV3a	AMA1/CSP	IIa	UK	Pevion (Thompson et al., unpublished data)
	Sporozoites, merozoites	Adenovirus 5	AMA1/CSP	I/IIa	USA	US Navy

Figure 4.2: Human malaria vaccines in recent or current clinical development. AdHu35, human adenovirus serotype 35; AMA, apical membrane antigen ; BPRC, Biomedical Primate Research Centre; CSP, circumsporozoite protein; FP, fowlpox; GLURP, glutamate-rich protein; GSK, GlaxoSmithKline; ME-TRAP, multi-epitope thrombospondin-related adhesive protein; MSP, merozoite surface protein; MVA, modified vaccina virus Ankara; NIH, National Institutes for Health; SSI, Statens Serum Institute; WRAIR, Walter Reed Army Institute of Research.

- the Malaria Vaccine Initiative (MVI) at PATH,
- the Bill and Melinda Gates Foundation and
- WHO (through TDR).

In addition, the African Malaria Network Trust (AMANET) and the Malaria Clinical Trial Alliance (MCTA) recently were established to build up the capacity to plan and coordinate malaria vaccine trials in Africa. It is to be noted that the recent infusion of public and private funding for malaria vaccine development has greatly accelerated the pace at which candidate malaria vaccines are entering the clinic. Increased collaboration and cooperation among key research stakeholders in the malaria vaccine field also has played a role in this trend. A recent international consultative process that engaged vaccine researchers, experts and donors has resulted in a Roadmap, which outlines a strategy for the development and licensure of an effective pediatric malaria vaccine by 2015 and a more broadly effective vaccine by 2025.

4.4 Usage of genetical modified mosquitoes

The usage of genetically modified mosquito that has refractory to transmission of the pathogen has been considered[15]. Recently, important technical advances, which include germ-line transformation of mosquitoes, the characterization of tissue-specific promoters and the identification of effector molecules that interfere with parasite development, have resulted in the production of transgenic mosquitoes incapable of spreading the malaria parasite[15]. However, in order for Plasmodium-refractory mosquitoes to be effective, they need to be able to thrive in the wild and compete successfully with their wild-type counterparts. One major concern about the use of these engineered mosquitoes is whether the modification would be stable long-term[15]. Even though the possibility of genetically modifying mosquito vector competence has been well studied in the laboratory, much work is still needed to develop strategies for the release and survival of these engineered mosquito populations in the field. In a recent study, it was reported that when fed on Plasmodium-infected blood, transgenic malaria resistant mosquitoes had a significant fitness advantage over wild-type mosquitoes[69].

In another application, interest had been in the possibility of converting mosquitoes from anthropophily to zoophily by the manipulation of their odour receptors. But generally still, the long-term fitness of these transgenic mosquitoes in totality and their ecological implications is not known. And the last straw, the political and social implications in the areas of human habitation are also other issues that will definitely stand tall to hinder deployment.

Recently, SIT (Sterile Insect Technique), one of the possible usages of nuclear radiated treatment, has been used to wipe out tsetse flies by a research team from the (The International Atomic Energy Agency, Vienna , Austria) in Zanzibar Island[82]. The methodology involved developing methods for mass rearing, improving sterilization and handling release technology for tsetse flies, and finally entomological and veterinary analysis to monitor the projects progress. Possibility of applying this technique to eradicate mosquitoes is currently being considered. In a personal communication, the International Atomic Energy Agency (IAEA) listed a number of hindrances as regards their capability to deploy genetically-modified mosquitoes in Nigerian cities and villages.

The SIT (Sterile Insect Technique) has been applied to several insects in different countries. In addition to its application to the eradication of tsetse fly in Africa - Zanzibar Island, SIT has already been successfully applied for fruit flies (USA and Latin America) and screwworm (USA, Libya). Feasibility studies for the control of the mosquito *Anopheles* albimanus and A. stephensi using SIT were conducted respectively in El Salvador and in India in the 1970s but not completed and the potential of the method is therefore unknown.

Our efforts at Covenant University

It has been noted[94] that a combination of new antimalarial drugs and vaccines with efficient vector control measures will be required to halt the transmission of malaria in the affected areas of the world. Evident is also the fact that from most part of Europe and north America, 1955 - 1969, malaria was eradicated using diligently a combination of Chloroquine (synthesized for example, into the household salt) and DDT with provision of infrastructures that allow application of DDT to breeding areas without undue exposure of DDT to man[58, 43]. At Covenant University, we are developing tools that will enable us to generate this kind of combination for deployment, starting with Nigeria at the National level. It is expedient to state here that one of the tools an evolution-proof insecticide is designed to be equipped with the effective capability of DDT but designed to target only malaria infected mosquitoes. This way, notwithstanding several breeding areas based on our struggling infrastructures, after deployment, we will soon have mosquitoes free of malaria.

For malaria research, irrational drugs design include the traditional search for a viable inhibitor compound, synthesized using medicinal chemistry or extracted from medicinal plant, the parasite is then druged via *in-vivo* and/or *in-vitro* experiment with the compound. To date, this kind of antimalaria treatment development has given birth to cinchona alkaloids, qinghaosu, chloroquine, chloroguanide (proguanil), amodiaquine, pyrimethamine, mefloquine, artemisinin and its derivatives and halofantrine[104].

The following illustrates drug design history (with emphasis on methods

and the drugs obtained via these) from pre-1960 till date:

- Pre-1960
  - **Serendipity.** chlordiazepoxide (librium), aspartame (sweetener), ether, acetylsalicylic acid, dicoumarol, etc.
  - **Clinical observations.** warfarin, LSD (hallucinogenic instead of cardiovascular activity), iproniazid (antidepressant instead of tuberculostatic activity), etc.
  - **Natural products.** quinine, taxol, morphine, digitaline, etc.
- 1960-1980
  - **Structure-Activity Relationships & screening.** Most modern drugs
- 1980-1995
  - **Rational design: ligand-based.** enalapril (high blood pressure and heart failure), cimetidine
  - **Rational design: protein-based.** HIV-1 protease inhibitors, TS (thymidylate synthetase) inhibitors (cancer therapeutics).
- 1995-present
  - Automated High-Throughput Screening (HTS)
  - combinatorial chemistry
  - genomics & proteomics
    in clinical development

Taking advantages of genomics (for the malaria parasite, *P. falciparum*[42]) and integrate-able data, such as the biochemical metabolic networks[23, 44, 57], microarray[64, 19], protein-protein interactions[62] etc, we have been able to encapsulate using bioinformatics approaches and thus rationally, the malaria's parasite (and recently mosquito) static and dynamic states. Novel and complex computational techniques[1, 3, 36, 37, 38, 73, 21, 5, 6, 59, 39, 75, 76, 77, 7, 8, 9, 10, 74] are then built to search for efficient antimalarial drug and insecticidal targets. Using medicinal chemistry[85] or plant directed by computational approaches[35, 87] (that include **virtual screening and**

docking[93, 101]), efficient inhibitors directed at these targets are developed (see the Figure below) and then tested biologically in an *in-vivo* and/or *in-vitro* experiment.

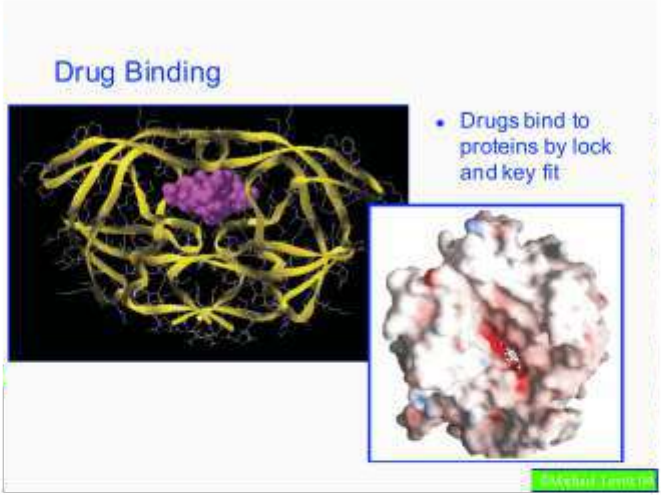


Figure 5.1: This picture shows how inhibitors bind to proteins.

The generation of the picture is similar to the fitting of a puzzle. This consists of the fitting together receptor (for example enzymatic site) and a ligand (inhibitor) by checking all relative receptor/ligand positions[61, 35]. This can be graphically illustrated using figure 5.2.

Success story in this direction is LuxS-a metalloenzyme involved in bacterial quorum sensing (a form of bacterial intercellular communication). LuxS is a target in Structural GenomiX pharceutical antibacterial therapeutic program. Figure 5.3a shows it bounded to the small molecule methionine. Figure 5.3b depicts the disphosphonomethyl group of one of Ariad pharceutical's compounds or drugs docked in the receptor binding site of Src SH2. The docking shown in this picture was made several months before the molecule was synthesized. Subsequent experimental data indicated that this predicted binding mode to Src SH2 was correct[18].

Further examples[32] include protease Thermolsyn and CDP inhibitor (see Figure 5.4(a)), HIV-1 Protease binding to a ligand (see Figure 5.4 (b)), Biotin binding to Streptavidin and Benzamidine binding to Trypsin (see Fig-

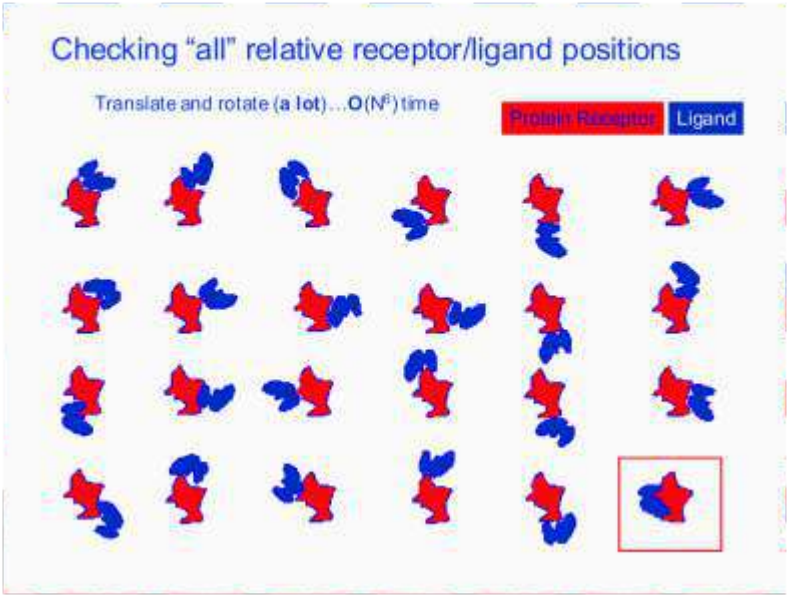


Figure 5.2: This picture shows how inhibitors bind to proteins computationally.

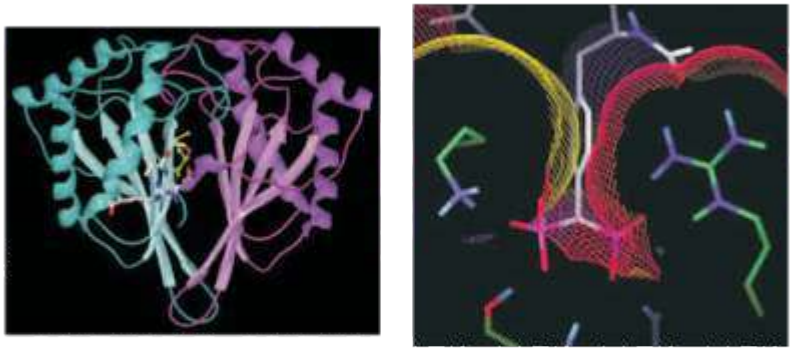


Figure 5.3: a) LuxS is shown bounded to the small molecule methionine. b) This picture depicted the disphosphonomethyl group of one of Ariad pharceutical's compounds or drugs docked in the receptor binding site of Src SH2

ure 5.5 (a)) and 7hvp shows JG-365 bound with the protease (see Figure 5.5 (b)).

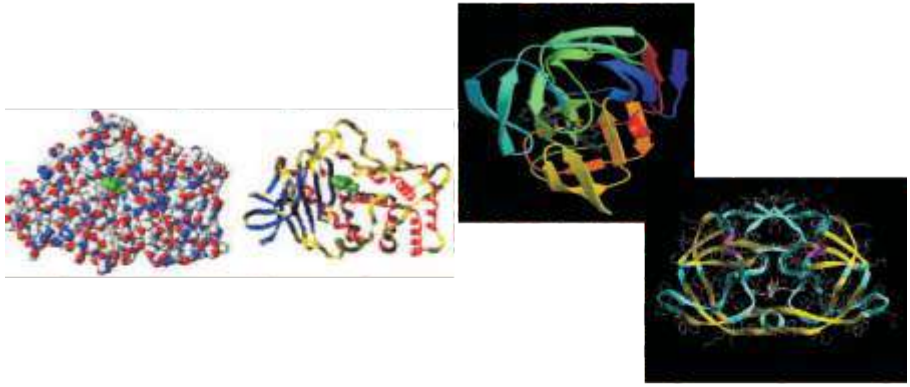


Figure 5.4: (a) protease Thermolsyn and CDP inhibitor, (b) HIV-1 Protease binding to a ligand

Figure 5.5: (a) Biotin binding to Streptavidin and Benzamidine binding to Trypsin (b) 7hvp shows JG-365 bound with the protease

Following the above steps, we are building very viable rational antimalaria treatments. The effectiveness of early diagnosis and prompt treatment as the principal technical components of the global strategy to control malaria is highly dependent on the efficacy, safety, availability, affordability and acceptability of antimalarial drugs. The effective antimalarial therapy not only reduces the mortality and morbidity of malaria, but also reduces the risk of resistance to antimalarial drugs. Therefore, antimalaria chemotherapy is the KEYSTONE of malaria control efforts. On the other hand, not many

new drugs have been developed to tackle malaria; of the 1223 new drugs registered between 1975 and 1996, only 3 were antimalarials[20]. Hence the need for a rational antimalaria treatment. In the light of the above, very advanced among our efforts is our antimalaria drugs development program. We hope to deliver via this program efficient, safe, readily available (apart from the search for synthetic antimalarial agents[39], we are currently also searching for bioactive compunds from "indigenous" medicinal plant[30]) and acceptable antimalarial drugs.

5.1 Bioinformatics in drugs development

Bioinformatics is an emerging field and is the modelling and application of computational techniques to solving **laborious** biology problems, begining with the like of proteins alignment to the more rigourous like of protein 3D structures prediction and molecular recognition of protein-ligand complexes.

Nowadays, the application of computer science to biology can be summarized in the following quotes: *"Computers are to biology what mathematics is to physics"- Harold Morowitz.* The following figure shows the various disciplines in bioinformatics.

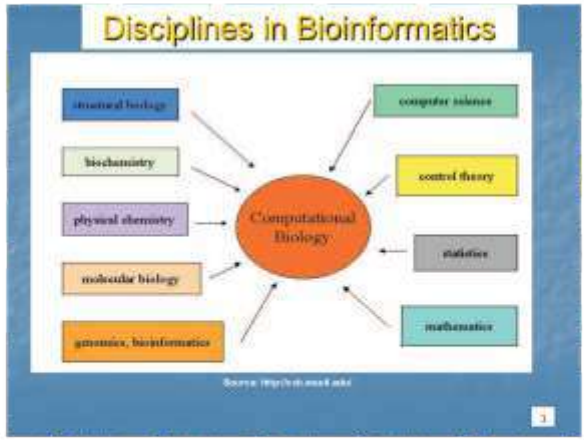


Figure 5.6: Disciplines in bioinformatics

When we talk of drugs here, we are looking at drugs meant for humans

and to rapidly kill malaria infected mosquitoes. We take a brief at discussing this from Roses[84].

It takes an average of 7-15 years to get a drug to market. From having the sites that encode potential targets for drugs (from our antimalaria drugs development program, enzymatic sites[36, 37, 38], signalling pathways[73], metabolic pathways[72] and transcription factors[21, 1, 5, 6, 11]) to actually having a medicine that interacts with that target involves a lengthy, expensive and complicated pharmaceutical pipeline that usually requires several years of basic science for target validation before chemical screening (see Figure 5.7 part a). Once there is a positive decision to progress a target, an effective screening assay that can allow the high throughput of many thousands of known chemical entities must be designed and implemented. Molecules that affect the target (hits) must then be evaluated for chemical properties and potency before those that are worth pursuing (leads) can be identified, synthesized, evaluated and modified for drug qualities (lead optimization (see Fig 5.8)). When a lead is identified, considerable pre-clinical development must also occur, particularly in the fields of toxicology, drug kinetics and drug metabolism. All of these processes must occur before the first dose of any new molecule can be tested in humans (see Fig 5.7 part b and Fig 5.9).

Following initial clinical testing for safety in humans, the molecule enters the most crucial phase, during which the desired clinical effect (that is, efficacy) is addressed in a relatively small, but still expensive, clinical trial. For example, if 100 patients participate in a Phase-IIA study at a minimum cost of US $10,000 per patient, the study would cost US $1 million. **In our program, the trial sites have been located in Nigeria, so cost is expected to drop drastically.** Even after a molecule demonstrates efficacy, more extensive dose-ranging studies, as well as studies, cost several hundreds of millions of dollars. With costs as high as this, only molecules for which there is good evidence of efficacy and a reasonable biological rationale for its mechanism of action are selected for full development.

5.2 Malaria control and eradication: A Bioinformatics approach

The overall goal of this project is to produce effective two high tech products for the control and final eradication of malaria starting with Nigeria.

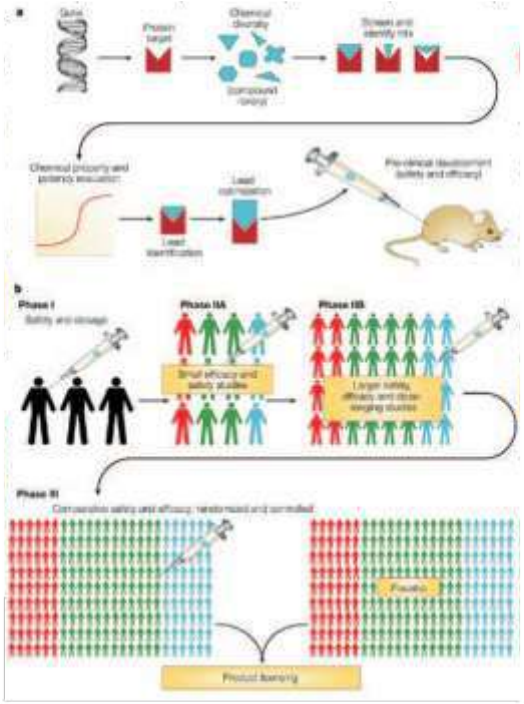


Figure 5.7: Taking a drug to market: Step-by-step

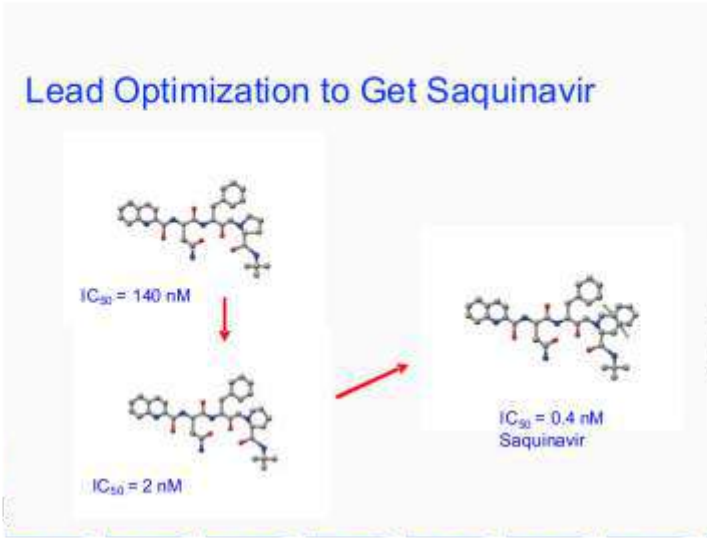


Figure 5.8: Lead optimization

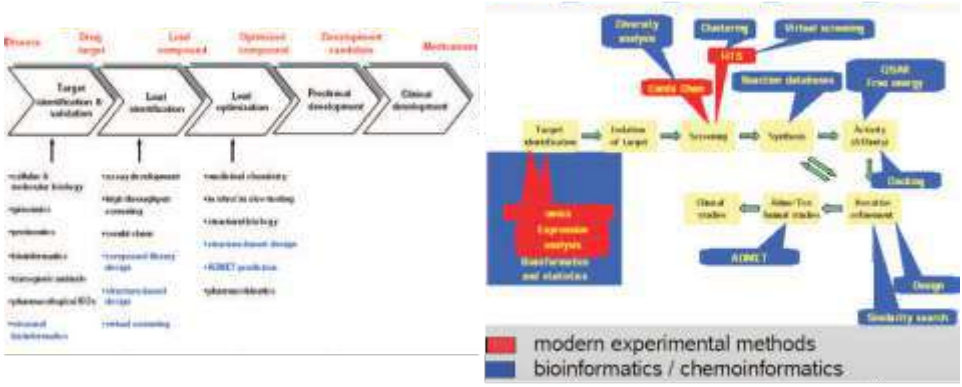


Figure 5.9: Role of bioinformatics in drugs development

The malaria parasite needs man and the mosquito to continue surving. For smartness of presentation, this is depicted in the figure below:

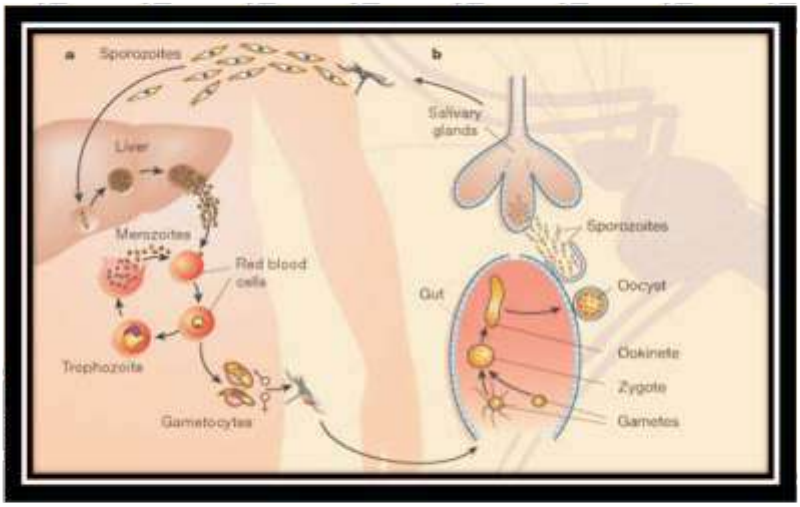


Figure 5.10: The complete life cycle of the malaria parasite in man and the mosquito[103].

Therefore, our first targeted product (from project I), **a cuisine of antimalaria drugs** (design and production cost is expected to be cheap, to enhance national large scale usage possibility, for example, should be embeded into the household salt) is to allow rapid cure of malaria in humans. This is to reduce to zero the chance of an uninfected mosquito to be infected after a bite. The second targeted product (from project II), **an advanced but human friendly pesticide DDT (a cocktail of agents)** is to help delete rapidly all malaria infected mosquitoes.

The expected result of the successful execution and application of our work will make Nigeria and eventually Africa, free of malaria infected humans and mosquitoes like the western world. This vision can can also be depicted in the following figure using the complete lifecyle of the malaria parasite in man and the mosquito[103]:

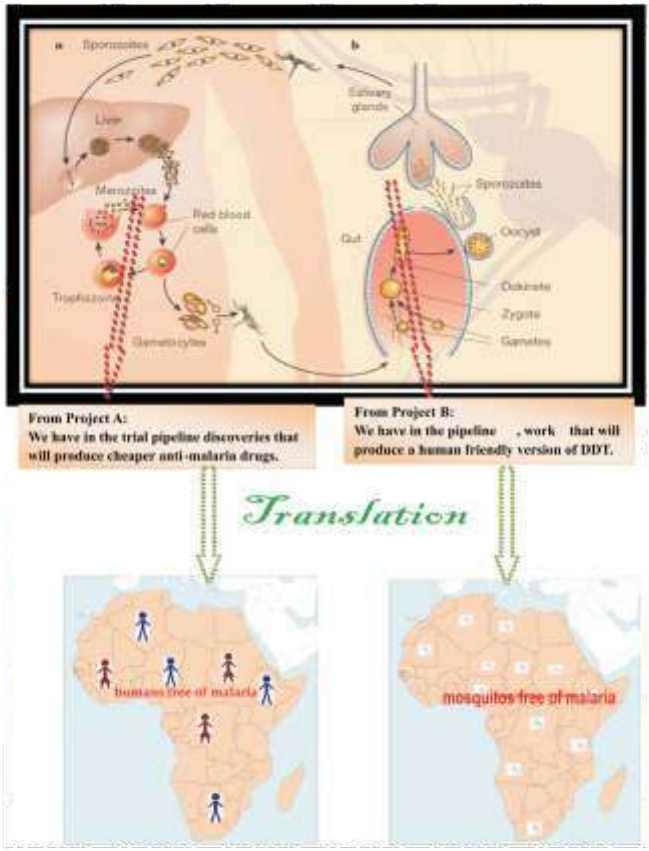


Figure 5.11: A Nigeria and eventually, also Africa, free of malaria, like the Western World.

5.2.1 Antimalarial drugs and diagnosing development

Please note, **our products move from three processing phases that include Bronze, Silver and Gold. At the latest (Gold) stage, the product is ready for large production and commercialization.**

A. Anti malarial drugs design and development (Silver):

The task of developing drugs (here, anti-malarial drugs) and taking them to the market can be depicted in the following Figure[84]. We indicated the stage we are in our development program via this figure.

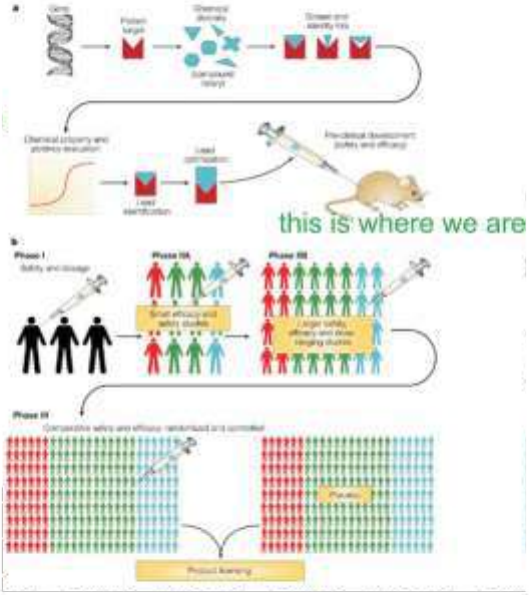


Figure 5.12: Taking a drug to the market.

To develop effective but cheap drugs against the malaria parasite, we are mining using novel *in-silico* techniques various drug target sites, namely, enzymatic sites[36, 37, 38], signalling pathways[73] and transcription factors[21, 5, 6]. Initial *in-vitro* antiplasmodial assay experiments in Prof. Michael Lanzer's laboratory at the University of Heidelberg, Germany[59] has been

very successful on some of the enzymatic sites listed in [36]. This proved that our *in-silico* techniques are correct and mined potential drug targets. Using our improved metabolic networks developed in [37], we have been able to mine more accurately four (4) enzymatic sites (not listed in [37] and the patenting of these potential drugs targets is in process) for which there are no known inhibitors (potential antimalarial drugs) to target them and for which the biological mode of actions of associated bioactive compounds will be entirely known. This discovery provides for the first time antimalarial drug target sites upon which a viable structural design pipeline is being built. And also provides a viable platform to optimize the fitting of "indigenous" medicinal plants bioactive compounds via a rational drugs design approach. Further pre-clinical development[39] is on-going to design and take successful inhibitors (drugs) to the market.

In the sections that follow, we give some techical detailing our exploration at arriving at the present results above.

In-silico mining of enzymatic antimalarial sites[36, 37, 38]

Aim, significance and formulation of the problem mathematically

In biomedical research, a considerable amount of data has been generated. Functional genomics of P. falciparum has been studied observed by the completion of sequencing the genome[42], a variety of gene expression studies[19] and the setting up of a comprehensive metabolic reaction database[115]. Methodologically, such a reaction database can be used to construct and systematically analyse a metabolic network by linking pairs of reactions for which the product of one reaction is the substrate for the other. Such metabolic networks have been analysed with graph-based algorithms to identify drug targets in pathogenic organisms. So the goal of our work here is to integrate genome-scale data based on metabolic networks for identifying essential genes in the malaria parasite, *P. falciparum*, that are new effective drug targets. The kind of genes we sought, can be depicted in the figure below:

Mathematical solution developed and results obtained

We used the data of the metabolic reaction database PlasmoCyc[55] and developed an algorithm that analyses the topology of the metabolic network for P. falciparum. Basically, each reaction in the network was deleted (knocked

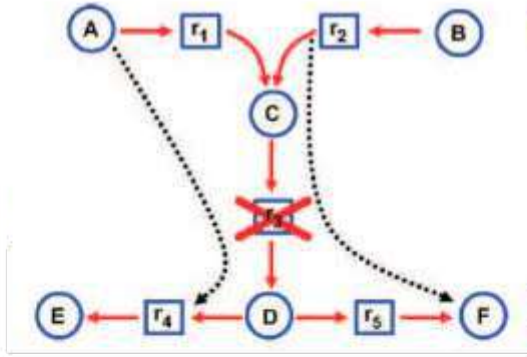


Figure 5.13: The knocked out reaction is a choke-point but may not be essential for the organism if the dashed lines exist in the metabolic network. Our approach inspects the network for such deviations. Reactions are illustrated as boxes, metabolites as circles.

out in silico), respectively. A breadth first search algorithm tested if the neighbouring compounds of the knocked out reaction could be produced by other reactions and pathways of reactions. In contrast to the choke-point approach, we checked the principle that deviations in the network could be used to replace the knocked out reaction. Fig. 5.2.1 illustrates this. The knocked out reaction is a choke-point, as metabolite D is uniquely produced by this reaction. However, D may not be essential for the organism if E and F can be produced using A and B as substrates for reactions r4 and r2, respectively (dashed lines in Fig. 5.2.1).

In the first instance[36], all metabolic reactions were extracted from the PlasmoCyc database([55], http://www.biocyc.org, Version 10.5). As described elsewhere[56] a connected graph was established by defining neighbours of reactions: two reactions were neighbours if a metabolite existed that was the product of one reaction and the substrate for the other. This yielded a bipartite graph of alternating reaction and metabolic compound nodes. Metabolites that were highly connected and therefore pathway unspecific, such as water, oxygen and ATP were discarded. This yielded a network with 554 metabolites and 575 reactions. As there was no information available, each reaction was considered to be reversible. Running our algorithm on the resultant bipartite graph yielded twenty-two (22) predicted drug targets

(enzymes).

In [59], we re-constructed a metabolic network from PlasmoCyc but restricting to enzymes for which genes were described either in PlasmoCyc, Malaria Parasite Metabolic Pathways (MPMP)[44] and HumanCyc[55], we built another metabolic network, **PMH**. Characterizing further topology properties and via the application of a new machine learning approach based on supprt Vector machine (SVM), we developed another approach for mining more effectively our desired enzymatic antimalarial drug targets. This is briefly encapsulated in the figure below:

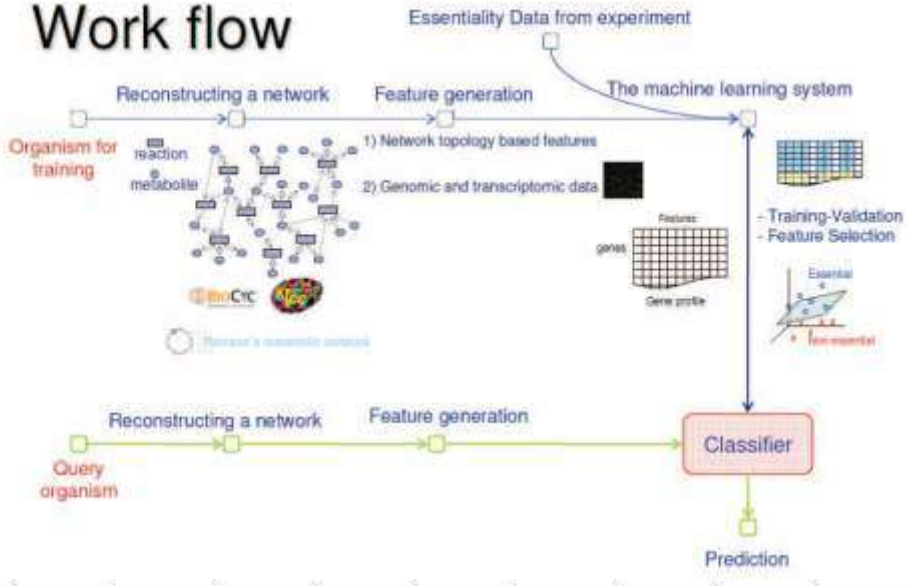


Figure 5.14: Machine learning based analyses on metabolic networks for identifying essential genes.

A test of our new approach on *S. typhimurium* yielded an excellent result as the essential genes predicted by our machine learning technique are the essential genes determined by an experimental screen. For the non-mevalonate pathway of *S. typhimurium*, our finding is shown below:

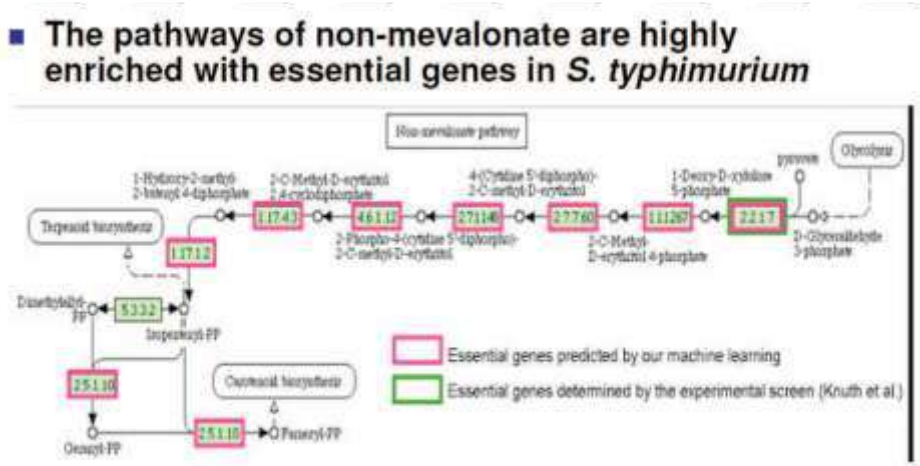


Figure 5.15: Computated essential genes versus experimental screening determined essential genes in the non-mevalonate pathway of *S. typhimurium*.

Using the above methodology on our new network, **PMH**, with the fore knowledge of the twenty-two (22) predicted drug targets (enzymes)[36], four (4) out of these twenty-two (22) enzymes were selected. Known inhibitor compounds were found for the first two enzymes, while there exist no known inhibitors for the last two. An in-vitro antiplasmodial assay experiments were performed on a Plasmodium falciparum Dd2 strain in Prof Lanzers lab, using one known inhibitor to target the first enzyme, while two known inhibitor compounds were used to target the second enzyme. Blocking the first enzyme resulted in successful death of the parasite, while the blocking of the second enzyme using these two compounds did not hamper significantly the well being of the parasite. These results are shown below in Fig. 5.16

Obviously further test is needed to evaluate the importance of this first enzyme in human but the experiments indicated the workability and correct prediction of potential drug targets by our computational technique.

In another further work[37], noting that there are some reactions defined in MPMP, which does not exist in PlasmoCyc, we extended our PMH network with these reactions using only the reactions, whose formulating compounds were listed on PlasmoCyc. We hope to include in the near future

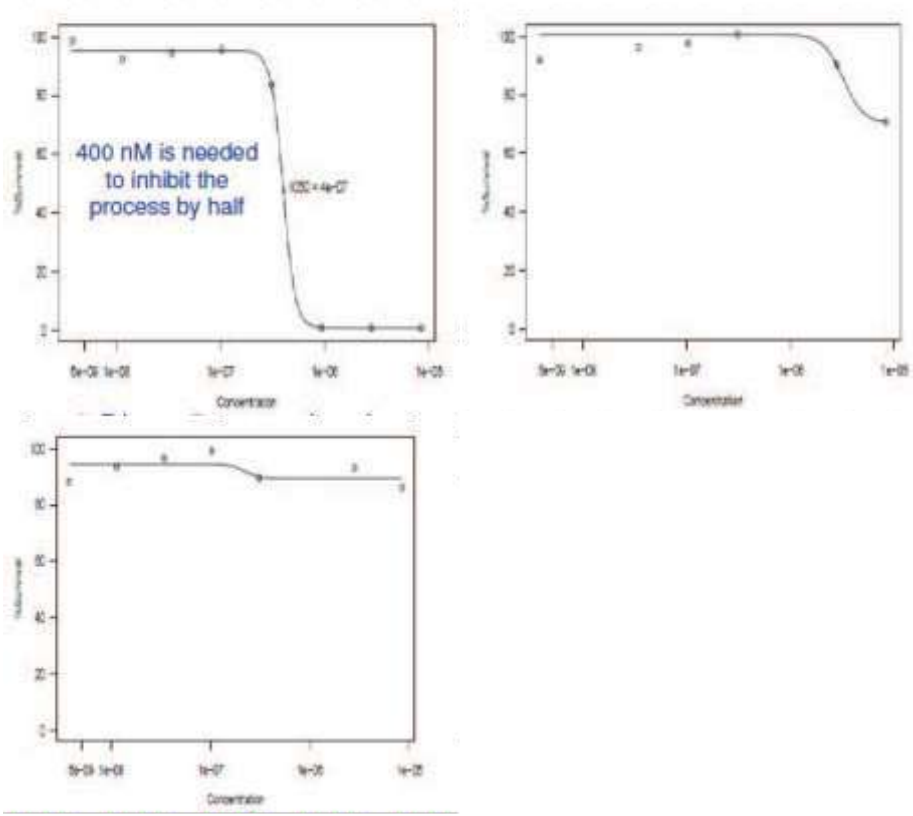


Figure 5.16: Blocking the first enzyme resulted in successful death of the parasite, while the blocking of the second enzyme using these two compounds did not hamper significantly the well being of the parasite.

the reactions that could be added now into our network after contributing to the updating of the PlasmoCyc. Running our computational analysis on the resulting network, PMH plus with other network variants (see Fig. 5.17 below), we obtained thirteen (13) predicted potential drug targets (enzymes). We observed that a number of dangling ends in the PMH network has been closed up by the addition of these reactions. Again, from these enzymes, all corresponding genes were compared to all proteins of the human genome using the web based BLAST[12] on the NCBI website.

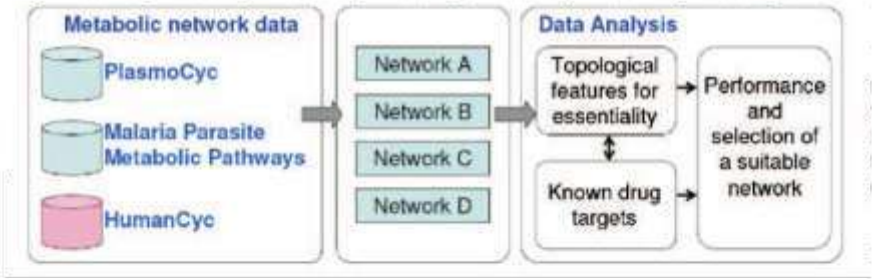


Figure 5.17: The workflow. Four reconstructed metabolic networks were compared (A-D). Network A contained computationally inferred reactions for Pf (from PlasmoCyc), Network B contained computationally inferred reactions for Pf and the human host (HumanCyc), Networks C and D contained only reactions for which enzymes the coding genes were known, C: network for Pf (information was extracted from PlasmoCyc and Malaria Parasite Metabolic Pathways), D: network for Pf and the human host. Topological features for estimating the essentiality of a reaction were computed for each reaction of these four networks. These predictions were compared with known drug targets and the consistency taken for estimating the performance of the network models.

Out of these thirteen (13) enzymes (not listed in [37]), we found four (4) enzymatic sites (the patenting of these potential drugs targets is in process)

- for which there are no known inhibitors (potential antimalarial drugs) to target them and
- for which the biological mode of actions of associated bioactive compounds will be entirely known.

This discovery provides

- **for the first time** antimalarial drug target sites upon which a viable structural design pipeline is being built.
- And also provides a viable platform to optimize the fitting of "indigenous" medicinal plants bioactive compounds via a rational drugs design approach.

Further pre-clinical development[39] is on-going to design and take successful inhibitors (drugs) to the market.

Computational identification of signalling pathways in the malaria parasite, *P. falciparum*[73]

Aim, significance and formulation of the problem mathematically

It is known[33, 52] that the ability to discover drugs or vaccine targets can only be enhanced from our deep understanding of the detailed biology of the parasite, for example how cells function and how proteins organize into modules such as metabolic, regulatory and signal transduction pathways. also noted is that the knowledge of signalling transduction pathways in Plasmodium is fundamental to aid the design of new strategies against malaria.

Biologically, a signal transduction pathway is the chain of processes by which a cell converts an extracellular signal into a response. In most unicellular organisms, the number of signal transduction pathways influences the number of ways the cell can react and respond to the environment.

Mathematical solution developed and results obtained

In Oyelade *et al.*,[73], we used a linear-time algorithm for finding paths in a network under newly constructed biologically motivated constraints to mine for the first time, chains of signal transduction pathways in *P. falciparum*.

To construct a protein-protein interaction network, protein protein interaction data was obtained from the work of LaCount et al.[62]. Their results comprise 2846 interactions between 1308 proteins of *P. falciparum* in its intra-erythrocytic cycle. In addition to the protein-protein interaction data, the transcriptional data from Le Roch et al.[64] and Bozdech et al.[19] were integrated to contributing to weighting the interaction reliabilities, depicted by the edges of the interaction graph.

The snapshot of our results is presented in Tables 1a-1e of [73] with the overall results given in Tables 2-8 of [73] in supplementary materials. From these results, we have been able to predict several important signalling transduction pathways in *P. falciparum*, namely,

- we have predicted a viable signalling pathway characterized in term of the genes responsible that may be the PfPKB pathway recently biologically elucidated by Vaid and Sharma[99] and Vaid et al.[100]. The figure in Fig. 5.18 below encapsulated this.

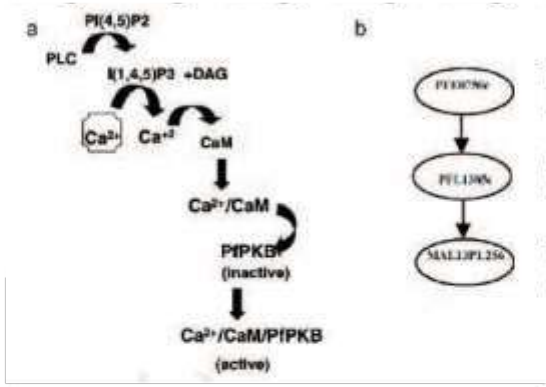


Figure 5.18: The Ca2+/Calmodulin-PfPKB signalling pathway as biologically dissected by Vaid and Sharma[99]. (b) The potential corresponding Ca2+/Calmodulin-PfPKB signalling pathway of Vaid and Sharma[99] from the protein protein interaction data of LaCount *et al.*[62].

- – We obtained from the FIKK family, a signal transduction pathway that ends up on a chloroquine resistance marker protein, which indicates that interference with FIKK proteins might reverse *P. falciparum* from resistant to sensitive phenotype.
  - We also proposed a hypothesis that showed the FIKK proteins in this pathway as enabling the resistance parasite to have a mechanism for releasing chloroquine (via an effux process)[54, 53].
  - Furthermore, we also predicted a signalling pathway that may have been responsible for signalling the start of the invasion process of Red Blood Cell (RBC) by the merozoites. It has been

noted that the understanding of this pathway will give insight into the parasite virulence and will facilitate rational vaccine design against merozoites invasion[70].

The predicted pathways are shown in Fig.5.19 below. A stage specific expression profile data for PFA0130c as obtained from plasmodb shows (see Fig. 5.20 below) that this serine protease protein is highly expressed at the ring stage for all the different cultures (HB3, 3D7, DD2) in Bozdech et al.[19] and two 3D7 (sorbitol and temperature) in Le Roch et al.[64] of the parasite used in experiments.

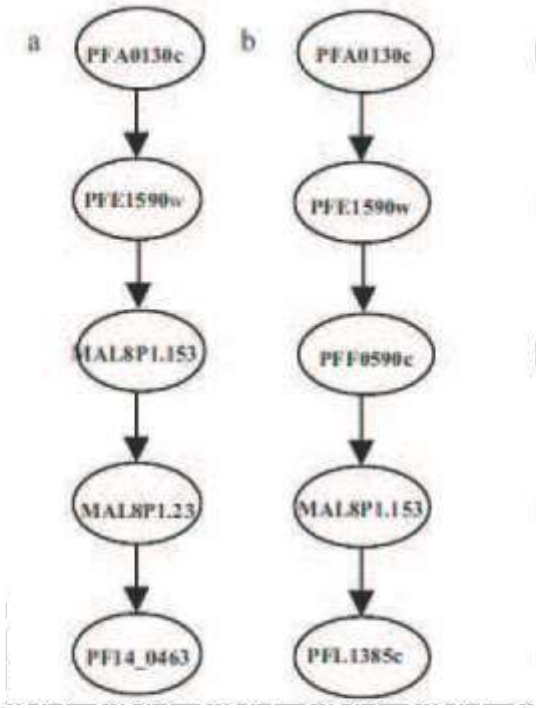


Figure 5.19: Potential vital signalling pathways from the FIKK family proteins as extracted into Table 1c. (a) Potential chloroquine resistance signalling pathway and (b) potential signalling pathway that may have signal the start of the invasion process of Red Blood Cell (RBC) by the merozoites.

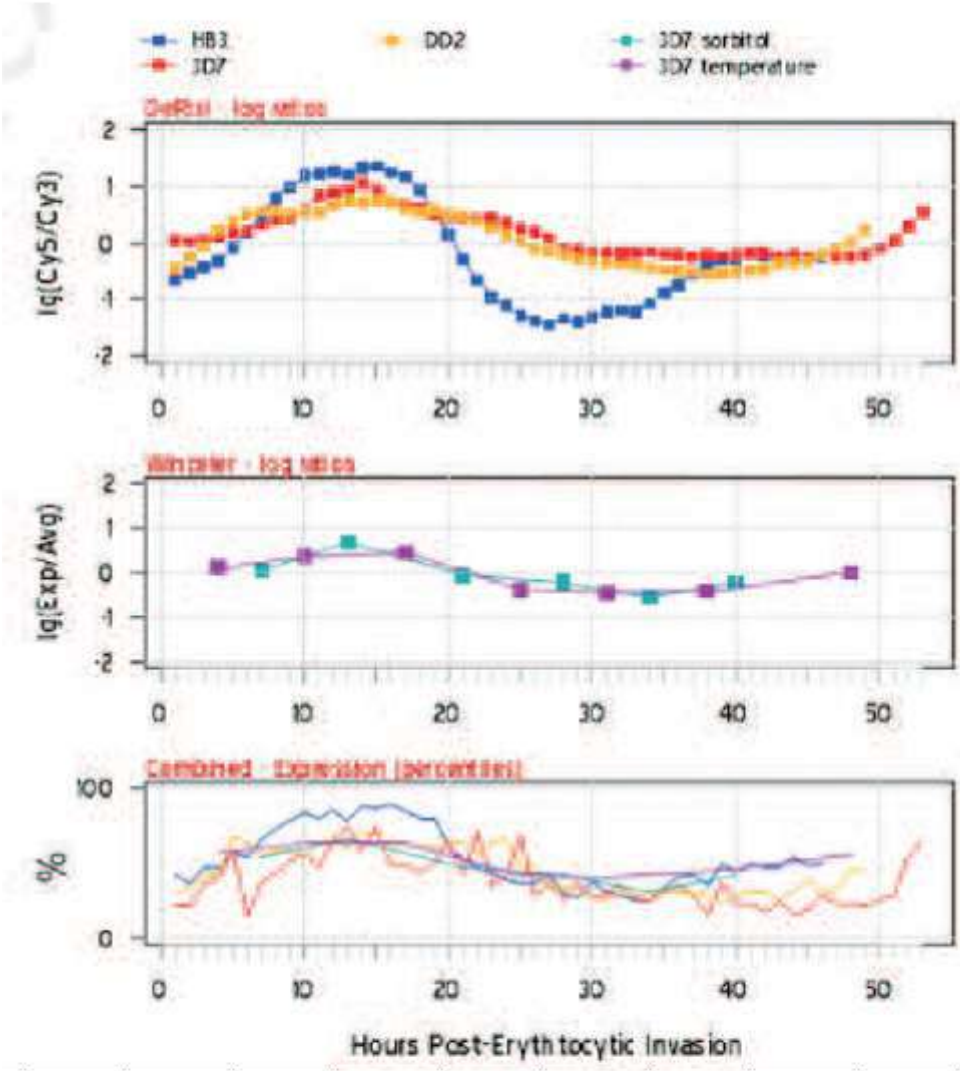


Figure 5.20: A stage specific expression profile data for PFA0130c as obtained from www.plasmodb.org

- And we have a host of other predicted pathways, some of which have been used in this work to predict the functionality of some proteins in the malaria parasite. Some of them are also depicted in the Figure 5.21 below.

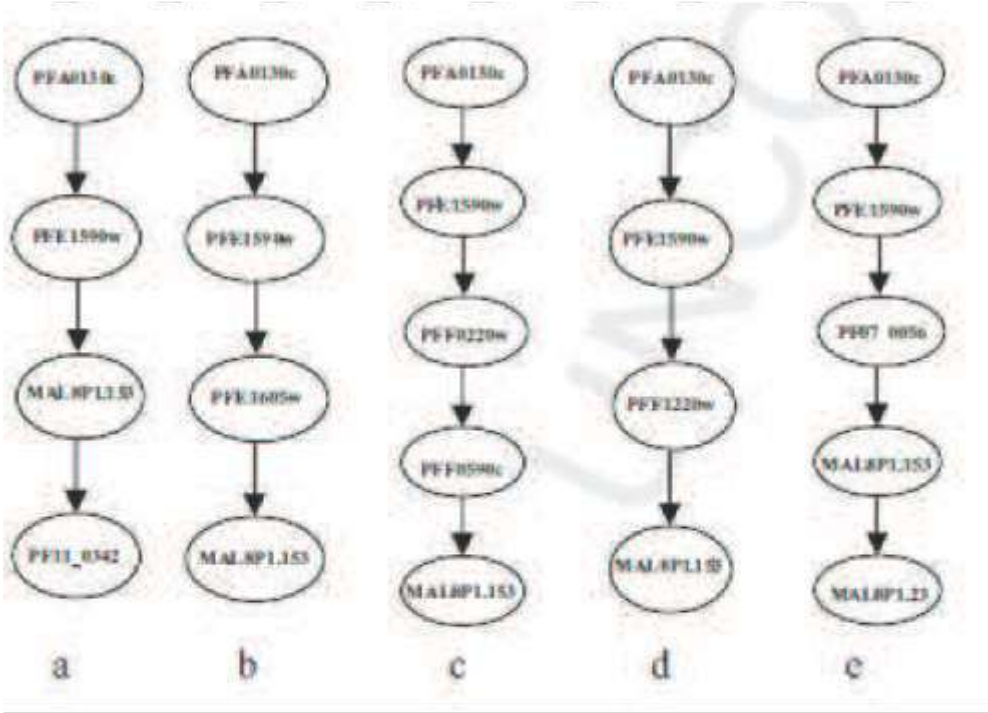


Figure 5.21: Hypothetical functional predictions from some predicted signalling pathways from the FIKK family proteins as extracted into Table 1a[73]. (a) P11_0342 was predicted to be a Merozoite Surface Protein, (b) PFE1605w, (c) PFF0220w and (d) PFF1220w as nucleus proteins and (e) PF07_0056 as a transcription factor.

Computational identification of metabolic pathways in the malaria parasite, *P. falciparum*[72]

Aim, significance and formulation of the problem mathematically

Metabolic pathways are processes by which the parasite produces the energy and components it needs to survive. The formally popular antimalaria drug Chloroquine, inhibit multiple sites in metabolic pathways leading to neutrophil superoxide release. Currently, the popular antimalarial drug artemisinin biological mode of action is controversial. Reports have shown that the parasite is growing resistance to existing drugs. Therefore, there is a renew effort to decipher clearly the metabolic pathways in *P. falciparum*.

Mathematical solution developed and results obtained

Here, we adapt the algorithm developed in Oyelade *et al.*, 2010[73] to extract linear pathways from *P. falciparum* metabolic weighted graphs (networks). The weights are calculated using the metabolite degrees and relevant pathways are obtained using atom mapping information. We also adapt and implement Scott et al., 2006[89] technique for extracting non-linear pathways.

Recently, we did an initial run of our algorithm (for four selected pathways: Pyruvate, Glutamate, Glycolysis and Mitochondrial TCA) on graph from KEGG and compare our results with the results obtained from current algorithms: ReTrace and atommetanet. Our results compare favourably with these two algorithms. Considering the results with genes classified into these pathways from Plasmodb, resulted into a lot of false positiveness. Furthermore, we compare the runs of our algorithm on graphs from KEGG and PlasmoCyc (from BioCyc). The results are remarkably different and the results from PlasmoCyc produce less false positiveness when compared to the results from Plasmodb. We identify 2, 1, 2, 4 gene(s) in addition to belong to these pathways respectively. Some of the genes have not been classified earlier to any known metabolic pathways

The goal in this project is to produce enzymatic pathways that are possibly not found in humans for our antimalarial drugs development program preclinical stage.

Transcription Factor(s)-Target Detection in the malaria parasite *Plasmodium falciparum*[6, 5, 2, 1, 21, 11]

Aim, significance and formulation of the problem mathematically

Recent review on the state of the art in malaria research can be found in Tuteja[94] and Ballou and Cahill[13]. And a perspective on the innovation application of systems biology to malaria research can be found in

Winzeler[105]. Since the 'Functional Genomics Workshop Group' meeting in Harvard, 2006, whose report was published in [34], several works[67, 41, 48, 116, 112] had attempted to solve many of the challenges identified by the group as key to bringing about the understanding of the biology of the deadly malaria parasite, *P. falciparum*. Key among the challenges to be overcome is: *identification of P. falciparum proteins involved in gene regulatory mechanisms*. Computationally, about one-third of the transcription factors (TFs) expected of the genome of the size of *P. falciparum* was mined in an earlier work[27, 16] but without the knowledge of the corresponding binding sites. Further works have also computationally mined binding sites from the DNA of *P. falciparum*[116, 49]. Although site-directed mutagenesis has validated the importance of some of these motifs controlling promoter activity[116], the transcription factors that bind to the DNA via these motifs have remained generally obscure[106]. Few attempts have been done to experimentally extract Transcription factors and their binding sites but these are still significantly marginal to what remains unknown[40, 118].

A result from Flueck *et al.*[40] depicted below, what is expected to be mined of Transcription Factor(s)-Target sites.

A review on the available biological process made so far as regards this and what to expect in the next ten years is contained in Horrocks *et al.*[47]. **The knowledge that these proteins interact with the genomic DNA to bring the genome to life; and that these interactions also define many functional features of the genome[50]**, which could be viable drug targets, makes mining transcription associated proteins (TAPs) (otherwise knows as transcriptional factors (TFs)) and their DNA binding sites a very important problem in malaria research. With this in mind, we have initiated and completed previous works[6, 5, 2, 1] targeted to give us an excellent leverage for the project expanded here. Briefly, our earlier works[6, 5, 2, 1] has focused on the extraction of simple motifs (these finds application in *prokaryotic* organism) and complex motifs (these finds application in *Eukaryotic*, *e.g.*, the malaria parasite, *P. falciparum*).

Mathematical solution developed and results obtained

In a step-wise apporach to reaching the goal of extracting in a large scale transcription Factor(s)-target in the malaria parasite *Plasmodium falciparum*, we did a microbial of what is expected in [21]. In this work, we provide computational insight into the regulation of *P.f.* genes in glycolysis and

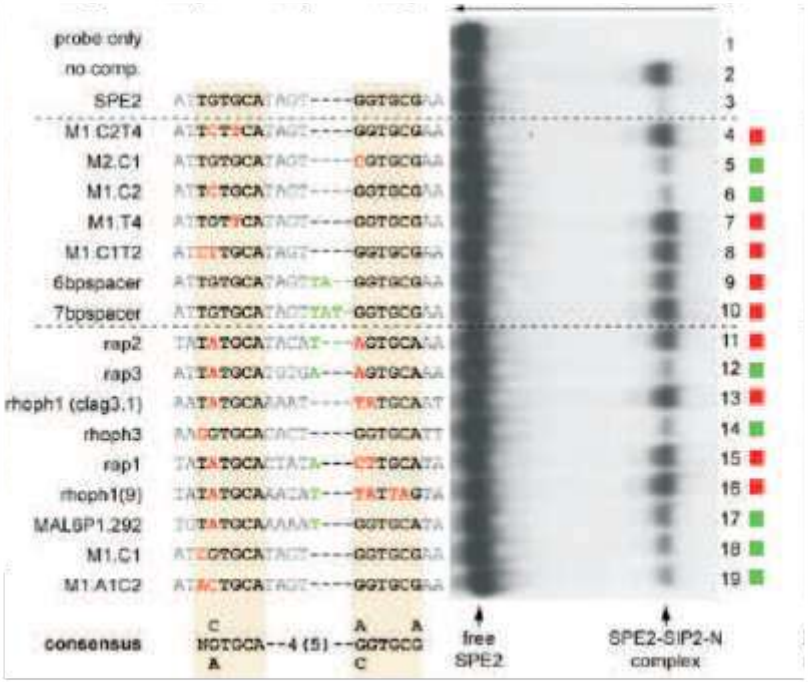


Figure 5.22: Competition EMSA using recombinant SIP2-N-HIS_B determined the minimal sequence requirements for binding of PfSIP2-N to a SPE2 consensus site. PfSIP2(PFF0200c from plasmoDB) is a transcription factor and above is its binding sites.

apicoplast pathways, in an attempt to elucidate the modalities of their regulation. Glycolysis is a crucial pathway in the maintenance of the parasite while the recently discovered apicoplast contains a range of metabolic pathways and housekeeping processes that differ radically to those of the host, which makes it ideal for drug therapy[88]. The time series data set used here is obtained from the work of Bozdech et al., 2003[19]. This data has been shown to compare very well with other existing data sets for Plasmodium falciparum and other Plasmodium species[117, 65]. For the glycolsis, from www.plasmodb.org, we harvested the twenty genes that are known to be involved at the glycolysis pathway and found eighteen (18) of these in the dataset of Bozdech et al., 2003[19]. We averaged the values from multiple oligonucleotides representing same gene. For the apicoplast, we obtained a complete list of twenty-seven (27) genes presently known to be involved from the DeRisi laboratory website and do the same as we have done for the glycolysis genes.

In another words, in these pathways, we attempt to explain the regulators involved and predict at the transcriptional level, the **transcriptional factors** responsible for their coordinated expressions. To do this, the following work-flow were designed, namely

1. modeling of regulatory logic using Bayesian inference and identifying potentially commonly regulated genes,

2. scanning their upstream regions (also taken from other plasmodium species) for commonly present, conserved sequence motifs by means of the PhyME algorithm[90],

3. computing a probability weight matrix from such motifs and scanning the genome of *P.f.* for further promoters that show these motifs.

Using a Bayesian model, for a time-series data, two different regulatory situations was formulated, namely 'simultaneous' ('OR'-model): the state of the gene i in the sample j depends on the states of its regulators in the same sample and 'time delay' ('OR-NOR'-learning): the state of the gene i in the sample j depends on the states of its regulators in the previous sample $j-1$.

For the glycolysis pathway, the results of these 'time delay' and 'simultaneous' learning are summarized in the following figure.

The 'OR-NOR'-learning mostly supported the results of the 'OR'-model but found some more activators and inhibitors with increased accuracy. The

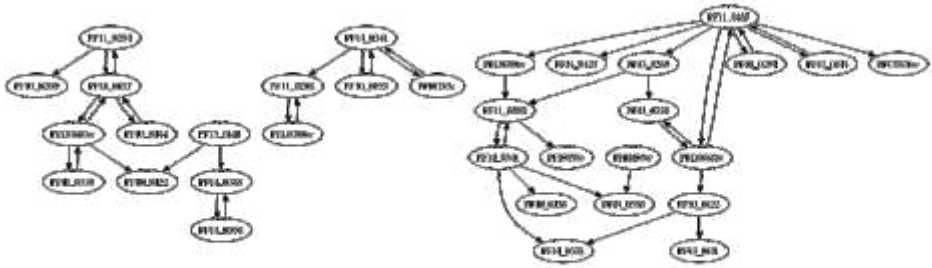


Figure 5.23: The results of these 'time delay' and 'simultaneous' learning on the glycolysis pathway.

regulatory network in figure 5.23(b) reveals the strategic position and hence the key regulatory role

of the genes PF11_0157, PFD0660w, PF14_0341 and PF13_0141. The inhibitory connections between the genes PFD0660w and PFL0780w, from the gene PFD0660w to the gene PFI0755c, and between the genes PF14_0425 and PF13_0144 might indicate three groups of genes working in timely separated manner. One group include the genes PF11_0157, PF13_0144, PF11_0294, PF13_0269 and PFD0660w. The second group contains the genes PFI0755c, PF14_0341, PF10_0155, PF13_0141, PF10_0122, PF14_0378, PFI1105w, PF14_0598, where the last three genes are closely connected with each other. The third group is: PF11_0208, PFL0780w, PFC0831w, PF14_0425 and PF11_0338. Using a query tool titled "Identify Genes based on Predicted Functional Interaction" from PlasmoDB, which is based on the data obtained from the work of Date and Stoeckert[29], the second group functionality or connectivity was overwhelmingly confirmed except for genes PF13_0141 and PF14_0378. We will suggest that a further biological studies should be carried out to check our prediction here. Furthermore, in group three, using this tool, we are able to show that genes PF11_0338 and PFL0780w, PFL0780w, PF11_0338 and PFC0831w and PFC0831w and PFL0780w are functionally connected. This tools could not verify the functional connectivity of PF11_0208 and PF14_0425 as we have shown theoretically. Based on the correctness of our predictions so far, we will suggest that these and group one connectivities (including their regulatory modalities) as shown here should also be tested biologically.

The genes PFD0660w, PF14_0341, PF11_0338, and PF14_0425 present interesting crosspoints between the separate groups. In the recent review

[114] that cataloguizes the various drug targets of P.f., three genes PF14_0341, PF13_0141 and PF14_0425 which encode three important energy metabolites, namely enzymes EC 5.3.1.9 (glycose-6 phosphate isomerase), EC 1.1.1.27 (lactate dehydrogenase) and EC 4.1.2.13 (aldolase) were stated as possible drug targets genes. Our theoretical finding predicts the important regulatory role of these genes in the glycolysis. The regulatory interactions of these genes to others reconstructed by our learning procedure should be verified in biological studies. Furthermore, biological literature supports our prediction that the gene PF14_0598 is been activated by PF14_0378 [79].

Fig. 5.23) (b) suggests the key regulatory role of the genes PF11_0157, PF11_0208, PF14_0341 and PF10_0155 and also reveals the groups of closely connected genes.

Interestingly, the gene PF10_0155 is connected to both enzyme genes PF14_0341 and PF13_0141. It was shown experimentally that the gene PF13_0269 is been activated by PF11_0157[97] as we have predicted here.

For the apicoplast, the results of these 'time delay' and 'simultaneous' learning are also summarized in the following figures.

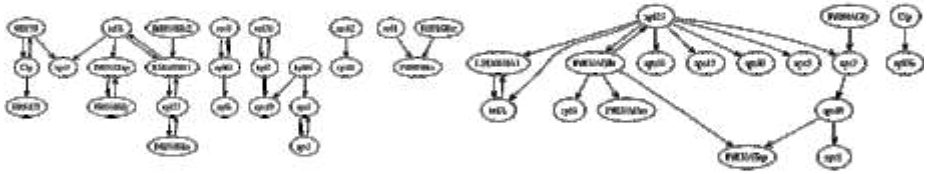


Figure 5.24: The results of these 'time delay' and 'simultaneous' learning on the apicoplast pathway.

One can see the strategical positioning of genes rpl23, ORF91, tufA, and rpl16. We also discovered from the literature[88] that these genes except ORF91 has been tested in wet lab experiments and have been marked as putative drug/herbicide targets. The observation of the 'time delay' regulatory interactions inferred further suggest the important role of Clp, rpl23, rpl16 but also of the genes rpl2, PtRNAGln and PtRNAThr. The gene rps19 was inferred to be regulated by many other genes. The graph of the 'time delay' 'OR-NOR'-regulation is much more connected than that of the 'simultaneous'.

We have been able to implement steps 2 and 3 of the work-flow designed above for PFL0780w, PF14_0425, PF13_0269, PF11_0294, PF13_0144, PFC0831w and PFD0660w, whose master activator has been predicted to be

PF11_0157. In our initial results, we have been able to show that a set of three motifs, compiled from seven genes (PFL0780w, PF14_0425, PF13_0269, PF11_0294, PF13_0144, PFC0831w and PFD0660w) potentially regulated by the same factor, is able to identify a number of other genes that harbor these motifs in their upstream region. Restricting to high-confidence presence of the motif, and requiring at least two of the three motifs to be present (simulating complex motifs), a set of seven genes (PFL1160c, PFB0480w, PFI0260c, PFI1605w, MAL8P1.143, PF14_0363 and PF14_0472) were found that show remarkable correlation of their expression values with those of the glycolysis genes used to compile the motifs.

In Adebiyi *et al.*[11], we have expanded on the work and results encapsulated above to facilitate, for the very first time, the *in-silico* discovery of transcription regulatory elements in *P. falciparum* using all its genes. Furthermore, the co-regulation of the genes, from which we mine these transcription regulatory elements, are computationally guaranteed instead of assuming that from the co-expression of these genes. Note that this assumption is not always true[116]. Our method also encapsulates a mechanism that guarantees the prediction of TFs and their binding sites. Finally, experiments shall be performed to validate these interactions.

B. PCR detection of malaria at the liver stage (Silver):

Waiting for the detection of malaria at the blood stage can lead to delayed treatment that may engender serious complication and death. The attachment of erythrocytes infected with Plasmodium falciparum to the microvessels of the brain leads to a pathological condition known as cerebral malaria that can result in death. There are no effective therapeutic means for alleviating this pathology[149] as adhesin proteins on the surface of the parasite-infected red blood cell aid malaria disease complication. It is therefore imperative to note that many lives will be saved if these parasites can be detected and treated at the asymptomatic liver stage instead of waiting till the disease manifestation at blood stage. In this chapter, we explored the basis of using PCR to detect malaria at the liver stage. Presently, we have developed computational systems biology techniques [75, 76] that has been used to mine viable genes that can be used to develop PCR experiments for the detection of malaria at the liver stage. Patenting of these genes are also in process. Our next task here is the development of PCR experimental protocols to experimentally validate the sensitivity of these genes[77].

We give details as regards progress made so far in this project.

Aim, significance and formulation of the problem mathematically

Malaria transmission involves three different developmental stages namely: the human liver stage, the human blood stages and the mosquito stage. Symptoms of malaria are expressed at the human blood stage. Generally, there is no doubt that there are some available drugs that can cure the diseases, but the problem in most cases is poor or late diagnoses resulting in complications and even death. The rationale for this study is to explore a diagnostic technique for detecting malaria at the liver stage, so that timely intervention can be made to alleviate the problem of the disease. Diagnostics on biochip has been making in-road into modern healthcare at a faster pedestal especially Point-of-Care than the lab-based diagnostics. The ultimate breakthrough may be to translate the result to a liver-based malaria diagnostics chip comparable to diagnostic chip used for detecting other diseases like HIV.

The use of microscopic examination of Giemsa-stained blood smears remains the cheapest and most commonly used method for the malaria diagnosis at the blood stage. Microscopy has its own bottle-necks in that its sensitivity is limited particularly when parasitaemia is low or when parasite morphology is altered. It is also time-consuming (Coleman et al., 2002) and requires a highly technical expert. There is no way to determine early invasion and infection since malaria diagnosis is carried out at the human blood stage of the parasite which accompanies the manifestation of the symptom for reported cases. Since the human liver stage is asymptomatic and precedes the blood stage, early diagnosis of the parasite at the liver stage will help intercept the havoc timely, through the use of some vaccines / drugs to abort the progression from liver stage to blood stage and thereby eradicate the malaria parasite at liver stage.

In-vivo experimental access to liver stages of human malaria parasites is practically prohibited and therefore rodent model malaria parasites have been used for *in-vivo* studies. However, genome-wide liver stage (LS) gene expression was profiled by using Green fluorescent protein-tagged *Plasmodium yoelii* (PyGFP) to efficiently isolate Liver Stage infected hepatocytes from the rodent host[150]. Our interest is to use this only known microarray information on liver stage of *P. yoelli* to find orthologue genes in P. falciparum, which can be used to develop a PCR experiment useful for the detection of malaria at the liver stage.

The compilation of the proteins that has been described to date as being relevant for the parasites survival and development in the hepatic stage is represented either in the micronemes/rhopteries or surface of the sporozoite[151]. These include:

1. Circumsporozoite (CS),
2. Thrombospondin-Related Anonymous Protein (TRAP),
3. Sporozoite Threonine-And Asparagine-Rich Protein (STARP),
4. Liver-Stage Antigen 1 (LSA-1),
5. Liver-Stage Antigen (LSA-3),
6. Sporozoite And Liver-Stage Antigen (SALSA),
7. Sporozoite Microneme Protein Essential For Cell Transversal (SPECT),
8. Spect2/*Plasmodium* Perforin-Like Protein 1 (PPLP),
9. Apical Membrane 3 Antigen/ Membrane Apical Erythrocyte Binding-like Erythrocyte Binding-Like Protein (MAEBL) and
10. P. falciparum Secreted Protein with Altered Thrombospondin Repeat (PfSPATR).

Generally, it should be observed that some of the proteins found in the hepatic stage of the parasite life cycle may also occur at the invertebrate host stage as well as the red blood cell stage. An investigator on one stage-specific protein that could be useful for PCR must first identify proteins that occur or are highly expressed at only one stage of the parasite development. Oyedeji *et al.*[152] identified three genes which express themselves at blood stage of malaria infection for their PCR-based comparative malaria diagnosis.

The limitations of diagnosing malaria by light microscopy of Giemsa-stained smears have led to the development of several new techniques[153] that aim to simplify and speed up diagnosis and increase sensitivity. Results have been obtained using fluorescent dyes (eg. with the quantitative buffy coat, QBC)[154] and simple dipstick tests to detect various antigens (World Health Organisation, 1996; Makler *et al.*[155]) as well as with PCR, regarded as the new reference method because of its superior sensitivity and specificity[152, 156].

In view of the limitation of Bruna-Romerio *et al.*[159], determination of important genes, is key before primers and probes can be produced for successful PCR that can be used for malaria detection at liver stage. Notwithstanding the importance of early and accurate diagnosis in malaria treatment discovery, most of the existing diagnostics gave little attention to malaria detection at the liver stage[78], hence the need to explore detection at this level of the parasite life cycle.

Mathematical solution developed and results obtained

Not much microarray work has been done on the liver stage of *Plasmodium* parasite. However, one promising microarray work was done by Tarun et al.[150] on the liver stage of *P. yoelli* parasite in mice. To deeply analyse the behavior of parasite genes at liver stage, we employed the use of the microarray data of Tarun *et al.* and searched for their orthologues in *P. falciparum* using the orthoMCL algorithm in PlasmoDB[157]. Our interest is to further analyse the behaviour of these liver stage genes using some knowledge obtained from blood stage of *P. falciparum* 3D7 and HB3 strains from the microarray data of Bozdech *et al.*[19]. This idea lends credence to the role of orthologues in functional genomics, as genes in a different species that evolved from a common ancestral gene by speciation, retain the same function in the course of evolution[158].

We searched PlasmoDB for the orthologues of 1985 *P. yoelli* genes represented on Tarun et al.[150] microarray. We obtained 1459 *P. yoelli* orthologues in *P. falciparum* 3D7 and HB3 strains. We mapped the expression values of these *P. yoelli* orthologues from Bozdech et al.[19] data and obtained 1180 genes for *P. yoelli* orthologue in 3D7 and 1163 genes for P. yoelli orthologue in HB3. 1139 genes were found to be represented in both *P. falciparum* 3D7 and HB3 strains. Traditional clustering algorithm implemented by us was deployed and used to cluster the 1985 genes of Tarun et al. microarray data, 1139 of *P. falciparum* 3D7 genes and 1139 of *P. falciparum* HB3 genes. We obtained the result in the table below.

Next, using R programming, we performed a Wilcoxon statistical test significance test to extract from these 139 *P. falciparum genes*, those that are highly similar (p-values ≥ 0.5). From these 139 genes, we found that only 54 are highly similar. We find the *P. yoelli* orthologues for these 54 genes using PlasmoDB and seek from Tarun *et al.* microarray expression data, the expression values of these orthologues at the liver stage. We compared

TRAD k-means Cluster ID	Clusters of P.y.elli from Tarun et al.		P.y orthologues in P.f 3D7 of Bozdech et al.		P.y orthologues in P.f HB3 of Bozdech et al.			
	No of Genes Present/ Cluster	Percentage of Genes Present (T1)	No of Genes Present/ Cluster (T2)	Percentage of Genes Present using the Total Gene clustered	No of Genes Present/ Cluster (T3)	Percentage of Genes Present using the Total Genes clustered	No of Genes Common to 3D7 & HB3	Percentage of Genes Present using the Totality of the Genes in this Category
1	167	8.41	140	12.29	156	13.70	63	45
2	64	3.22	112	9.83	77	6.76	37	19
3	81	4.08	29	2.55	52	4.57	1	1
4	213	10.73	19	1.67	52	4.57	0	0
5	192	9.67	52	4.57	70	6.15	3	2
6	57	2.87	58	5.09	13	1.14	0	0
7	137	6.90	149	13.08	21	1.84	3	2
8	226	11.39	114	10.01	93	8.17	1	1
9	142	7.15	14	1.23	54	4.74	3	2
10	107	5.39	42	3.69	24	2.11	0	0
11	65	3.27	111	9.75	74	6.50	31	22
12	65	3.27	34	2.99	197	17.30	2	1
13	148	7.46	11	0.97	110	9.66	0	0
14	166	8.36	104	9.13	103	9.04	1	1
15	155	7.81	150	13.17	43	3.78	4	3

Figure 5.25: Comparative Table for *P. yoelli* orthologues in *P.falciparum*. The table illustrates the *P. yoelli* orthologues in both 3D7 and Hb3 strains with their various percentage score. Column 9 indicates the number of common genes in two clusters with the same ID number from 3D7 and HB3 strains. Relatively high percentage score was obtained for cluster 1, 2 and 11 giving an indication that each of these 3 clusters is closely similar in both strains (3D7 and HB3) and may make a good choice for a gene to be used for PCR detection of malaria parasite at liver stage. More explanation is given for these genes in column 10 as to the choice of PF13_0227, PFL1700C, PF13_0227 and PF13_0358. 139 genes were found to be in corresponding clusters for both 3D7 and HB3.

the expression values of the 53 genes at the liver and blood stages. The idea behind this is that the genes that have highly dissimilar gene expression at the liver and the blood stages will be the theoretical/statistical viable candidates. Doing this we arrived at 29 genes set. Due to intellectual property right, we will not be able to list these genes.

5.2.2 Development of an evolution-proof insecticide

Aim, significance and formulation of the problem mathematically

It is to be recalled in section 4.2 that there is a new renewed effort to identify new insecticidal compounds for use in malaria control[46, 119]. This is particularly urgent based on the rate at which resistance is emerging against available compounds, noting in addition that presently, insecticides recommended for malaria control by the World Health Organization (WHO) represent just four classes of compound for IRS and just one class of compounds for ITNs[71, 25].

Noting the success in the eradication of malaria from the North America, Europe and Australian via the use of DDT, the purpose of the project is to identify and validate evolution-proof insecticidal targets in *A. gambiae* mosquito. Put in the language of DDT, we aimed to design a modified Dischlorodipheytrichloroethane (DDT) that will not harm humans and vital species and will be devoid of any resistance mechanism attempt of the mosquitoes. Strong non-genomics rationals behind such innovation have been carefully enumerated in a Read, Lynch and Thomas, 2009[83]. The targets in *A. gambiae*, we hope to discover, will only be valid for it, when it carries the malaria parasite. This will help us in a later project, to deliver insecticidal compounds that will contribute to the truncation of the malaria parasites development within the mosquito mid-gut. This will utimately lead to the reduction of major occurrences of malaria parasite transimissions and invariably, we strongly believe, to the final eradication of malaria.

More formally, the overall project goal is identification and validation of evolution-proof insecticidal targets in *A. gambiae* mosquito. This was broken down into two problems, namely

- *In-silico* study of the complex mechanisms of mosquito insecticide resistance and

- *In-silico* identification of genes or pathways that are differentially regulated when the malaria parasite is going through its life-cycle within the mid-gut of the *Anopheles gambiae*.

The indicators of success in this work for the two problems above are

- List of clusters (pathways) of genes predicted to execute resistance mechanism in the mosquito and
- refined list of insecticidal targets with elaborate comparison with targets published already in the literature.

We will evaluate the above indicators using the following experimental validations:

- Validation in *A. gambiae* using RNAi gene silencing and
- validation infected *A. gambiae* and selection of targets which kill the mosquito.

We are presently extending a number of our techniques in [7, 36, 37, 38, 21] to find applications in the challenges of this project.

It is important to give a brief here that have informed us on a number of fronts, we are presently pursing in the development of techniques for the challenges here. In Adebiyi *et al.*[7], we pursued elucidating the drugs resistance mechanism(s) of the malaria Parasite to tetracyclines, chloroquines and T4 choline. The model developed in this work include the capacity to analyse gene expression data on metabolic networks. The model include:

- Construction of the metabolic network,
- Network clustering,
- Mapping gene expression data onto the reactions,
- Feature extraction and
- Analysis of stimulated and repressed pathways.

The results (extracted stimulated and repressed pathways) obtained applying this model to the analysis of the gene expressions of *P. falciparum* over its metabolic network, although interesting, is not statistical significant.

Mathematical solution developed and results obtained

Based on the results (mostly averagely significant) from our earlier work in [7], via the computational pipeline above, more work is presently ongoing to produce more effective novel computational techniques. In the next one year, we hope to complete the insecticidal targets discovery and move on to the preclinical testing stage of the program.

5.2.3 Computational modeling of an *Anopheles gambiae* metapopulation for malaria control

Aim, significance and formulation of the problem mathematically

One of the major methods to control and possibly eradicate malaria is by controlling this vector insect. While traditional control methods have significant value (like DDT), there is much recent interest on the use of novel control technologies based on genetic manipulation of the mosquito or its associated symbiotic microorganisms. For example, Sterile Insect Technique (SIT) has been applied against tsetse and fruit flies and screwworm. Possibility of applying this technique to eradicate mosquitoes is currently being considered. In a personal communication, the International Atomic Energy Agency (IAEA) listed a number of hindrances as regards their capability to deploy genetically-modified mosquitoes in Nigerian cities and villages.

Mathematical models are a crucial part of novel control techniques, since they are one of the only ways to optimize deployment and conduct risk-assessment prior to an actual release. The aim of this research is to create a spatially-explicit, stochastic model of an *Anopheles gambiae* metapopulation to computationally model the population dynamics population genetics of this deadly vector. Once this is completed, novel control strategies, such as the release of genetically-modified mosquitoes or symbiotic bacteria or viruses will be added to the model.

Mathematical solution developed and results obtained

Due to a number of mutual understanding and collaborating constraints, I will not (at this point) be able to give details on our progress in this presentation.

Conclusion and Further-work

The CODE MALARIA will be ready for full deployment after the completion of the two targeted product (from project I), a cuisine of antimalaria drugs and an advanced but human friendly pesticide in the version of DDT (a cocktail of agents) (from project II). Backup therapy during deployment include various models that will empower us to deploy for example SIT technology against the mosquitoes if the need to do so arises.

We also have a work on-going in computational vaccinology. The aim of this new work is to tackle various limits[28] hindering the active application of computational vaccinology to vaccines development especially in the development of anti-malaria vaccine(s).

The final goal is to ensure that in this decade, Africa and infact, wherever malaria is a menace, this becomes a forgotten headache as we presently have in the western countries.

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