medicinska revija
medical review

UDK: 616.936-074:577.112
COBISS.SR-ID 212930572

Caceres Hernandez J. L. et al ■. MD-Medical Data 2015;7(1): 007-014

Originalni članci/
Original articles

APPLICATION OF THE RESONANT RECOGNITION MODEL TO THE STUDY OF PLASMODIUM PROTEINS INVOLVED IN MALARIA INFECTION*

PRIMENA MODELA REZONANTNOG PREPOZNAVANJA U ANALIZI PLASMODIUM PROTEINA KOJI SU UKLJUČENI U MALARIČNU INFEKCIJU*

Jose Luis Hernandez Caceres[1], Irena Cosic[2,3], Drasko Cosic[3]

[1]School of Medicine and Allied Health Sciences, University of The Gambia
[2] RMIT University, Melbourne, Victoria, Australia
[3] AMALNA Consulting, Black Rock, Victoria, Australia

Correspondence to:

Irena Cosic, Emeritus Professor, RMIT University Director, AMALNA Consulting,
46 Second Street, Black Rock, 3193 Victoria, Australia
www.amalnaconsulting.com
e-mail: irenacosic@me.com

*Invited paper / Rad po pozivu

Abstract

Invasion of the human host by plasmodium parasites involves complex interactions with key roles played by both human and plasmodium proteins. Here, using signal processing method, Resonant Recognition Model (RRM), we analyzed a group of proteins playing key roles in these invasion mechanisms. The RRM is a physico-mathematical approach analyzing protein interactions based on resonant frequency match between free electron energy distributions of interacting proteins. By identifying resonant frequencies common to several proteins involved in plasmodium infection, the main features of invasion process can be summarized. The findings of this study confirmed results from literature: interaction of band 3 protein from Red Blood Cell with both Erythrocyte Binding Antigen (EBA) and Signal Peptide Protease (SPP), following different mechanisms, common function for orthologues of Circumsporozoite protein, EBA, SPP and PfEMP. Two main characteristic frequencies were identified as relevant for parasite invasion: frequency at f=0.0020 is putatively associated to recognition of host cell membranes, whereas frequency at f=0.3400 may reflect intracellular interactions of merozoites as well as hypnozoites. The results obtained here provide a valuable insight into protein-protein interactions during plasmodium infection which could be used further to design new synthetic peptides as vaccine candidates or medications for curbing malaria.

Key words
Malaria, Circumsporozoite Protein, Erythrocyte Binding Antigen, Band 3 erythrocyte membrane, *Plasmodium falciparum, Plasmodium vivax*, Resonant Recognition Model, Protein-to-protein interactions

Ključne reči
Malarija, Circumsporozoite Protein, Erythrocyte Binding Antigen, Band 3 erythrocyte membrana, *Plasmodium falciparum, Plasmodium vivax*, Model Rezonantnog Prepoznavanja, Protein-protein interakcija

INTRODUCTION

Malaria is one of the most ancient diseases. Human populations strived to cope with it, sometimes via health-compromising mutations in endemic areas: Gerbich blood group antigen negativity[(1)]; polymorphisms of the complement receptor genes; Southeast Asian ovalocytosis[(2,3)]; pyruvate kinase deficiency[(4)]; haemoglobin E[(5)]; the sickle cell trait[(6)]; α-thalassaemia[(7)] and sometimes tensioning indigenous knowledge up to their best performance levels. Indeed, quinine derivatives and artemisinin derivatives are today's main weapons against malaria. The name quinine comes from the Amerindian word for the cinchona tree *Cinchona ledgeriana*, quinaquina, which means „bark of barks". *Chinchona* was used for centuries in the Ands by local populations to combat fever and Artemisinin is coming from *Artemisia annua*, a plant used as an ancestral malaria remedy in China[(8)].

Malaria is caused by different species of Plasmodium which has two hosts: Anopheles mosquito and human. *Plasmodium* species represent an extremely successful group of parasites that can efficiently infect their hosts by

rapidly gaining entry to cells using their own invasion apparatus to identify, penetrate and establish themselves. *Plasmodium* is transmitted to human hosts by female *Anopheles* and sporozoite forms are injected during a blood meal. In the liver, sporozoites pass through a number of host cells before invading hepatocytes, where they differentiate and divide into merozoites that, when released, invade erythrocytes, thus beginning the asexual blood-stage lifecycle. Merozoite's polarized morphology and presence of apical organelles that secrete their contents during host cell invasion are essential in their strategy for gaining entry into red blood cells (RBC).

Among the *Plasmodium species*, both *P. falciparum and P. vivax* have evolved a highly complex process for the invasion of erythrocytes with multiple steps and various parasite ligands that mediate invasion through redundant pathways. This elaborate molecular invasion machinery ensures the ability of *P. falciparum* to invade erythrocytes of all ages. The redundant invasion pathways also guarantee that *P. falciparum* does not depend on any single molecule on the erythrocyte surface for invasion, as is the case for *P. vivax*, which requires the Duffy blood group for invasion, while preferring small-sized erythrocytes[9]. Malaria caused by *P. vivax* has been drastically reduced in many parts of Africa due to the high prevalence of Duffy blood group null erythrocytes among the population. At the same time, *P. vivax* develops a dormant stage inside the hepatocyte (hypnozoite) that is responsible for malaria relapses in *vivax*-endemic areas. Preference of *P. vivax* for reticulocytes poses additional challenges to both malaria treatment and research[10].

During invasion, specific attachment of merozoites to erythrocytes is followed by reorientation and junction formation. So far, the molecular basis for these processes remains unknown. Various parasite ligands have been identified as role players, often with a high degree of redundancy.

One approach to creating a malaria vaccine is to target proteins that are known to play roles in the invasion of erythrocytes/hepatocytes. However, the fact that none of the 27 vaccine candidates, in study in 2013, has met so far the target set by World Health Organization of more than 75% protection, might illustrate how far we are from a deep understanding of malaria's molecular mechanisms[11]. Difficulties, however arise not only from our limited knowledge as *Plasmodium* is equipped with very efficient immunological escape mechanisms that make the parasite transparent to the responses of the immune system[12], as well as resistant to the action of quinolones and artemesinin[13].

The absence of clear breakthrough in malaria combat could support the need for different ways of tackling the disease that are supported by conceptually new bases. Among these, an approach based on the Resonant Recognition Model (RRM) can be promising. The RRM is a unique model which proposes that protein-protein, protein-DNA and protein-RNA interactions are based on resonant recognition with unique characteristic frequency for each interaction[14-16]. The RRM is based on finding that each biological function/interaction of protein is branded by specific periodicity/frequency in distribution of free electron energy along the protein. In particular, two interacting proteins will have the same resonant frequency, but opposite phases. Mounting experimental evidence is supporting the idea of RRM as a universal mechanism of protein-to-protein interaction[14-16]. In particular, mutated proteins that keep their biological activity will preserve the resonant frequency and RRM-based putative ligands might be robust to the presence of immune evasion[17].

Here we describe the most salient outcomes of an application of the RRM to the study of a group of proteins that have been proposed as promising vaccine candidates due to their crucial role in *Plasmodium* infection mechanisms. Our results seem to be encouraging for suggesting RRM-based strategies for combating malaria.

MATERIALS AND METHODS

Resonant Recognition Model

The RRM postulates that protein/DNA/RNA interactions entail a mechanism of resonant energy transfer between involved molecules at the frequency specific for each observed function/interaction[14-16]. This is the universal mechanism by which macromolecules and smaller peptides recognize each other. A key aspect of the RRM approach is to represent a protein's primary structure as a numerical series. For this, each amino acid in the sequence is symbolized with the (numerical) value of a biologically relevant physico-chemical parameter. If the chosen parameter is suitable, it happens that proteins with the same biological function have a common frequency component in their Fourier spectra. This common frequency is considered to be a hallmark of a protein's biological function or interaction.

In this work, the numerical sequence is obtained by assigning to each amino acid, the energy of delocalized electrons, calculated as the electron–ion interaction pseudopotential (EIIP) of each amino acid residue. The EIIP parameter describes the average energy states of all valence electrons in a particular amino acid. Accordingly, the resulting numerical series represents the distribution of the free electron energies along protein's backbone.

Once the numerical sequence is obtained, it is submitted to spectral analysis using the Fourier Transform (FT) to extract information pertinent to the biological function. In the frequency domain, the FT of an individual protein sequence will contain nonzero values for many frequencies. However, if a cross spectral function is estimated for a group of proteins sharing one common frequency, the cross spectral function will have a nonzero value at this resonant frequency. Since it is common that a given protein can display more than one function, it may happen that the cross spectrum of a group of orthologous proteins will exhibit more than one peak. This is to be expected for the *Plasmodium* sequences studied in this work, since some of them exhibit more than one function/interaction.

The multiple cross-spectral function for a large group of orthologous sequences with the same biological function has been named 'consensus spectrum'. The presence of a distinct peak frequency in a consensus spectrum implies that this common frequency is related to the shared biological function provided the following criteria are met:

(1) One peak only exists for a group of protein sequences sharing the same biological function;

(2) No significant peak exists for biologically unrelated protein sequences;

(3) Peak frequencies are different for different biological functions.

The study of a great diversity of proteins has provided support for the conclusion that each specific biological function of a given protein or DNA is characterized by a single frequency[14-18,38-42]. The RRM can be applied to the study of interactions of proteins with their targets (receptors, ligands and inhibitors) since it was found that interacting proteins and targets display the same characteristic frequency in their interactions.

Thus, the RRM characteristic frequencies represent a protein's general functions as well as the mutual recognition between a particular protein and its target (receptor, ligand, etc.). As this recognition arises from the matching of periodicities within the distribution of energies of free electrons along the interacting proteins, it can be regarded as the resonant recognition.

It has been found that peptides attaching to proteins do share the same resonant frequency and exhibit opposite phase[14-18,38-42]. Thus, abiding these two conditions is regarded as a hallmark for protein-protein interaction.

Sequences

We centered our study using RRM analysis on finding consensus spectra for groups of similar proteins involved in parasite infection of red blood cells/hepatocytes, as well as some of the most important vaccine candidates reported in literature.

The following protein groups were analyzed:

Circumsporozoite protein (CS)

Plasmodium sporozoites are inoculated into the human host by the bite of an infectious mosquito. Sporozoites pass through the cytosol of several cells before invading a hepatocyte, process ascribed to the interaction of CS with proteoglycans on the surface of the hepatocytes. Anti-CS antibodies have been shown to inhibit parasite invasion and are also associated with a reduced risk of clinical malaria[19]. The only phase-3 vaccine for malaria so far is based on CS[20].

The CS was the first well-studied example of a repetitive malarial antigen. The CSs of all malaria parasites studied so far comprise central tandem repeats that form immunodominant B-cell epitopes. The *P. falciparum* CS, for example, contains 37 to 50 copies of 4-mer motifs, but relatively short repetitive synthetic peptides are thought to express the full antigenicity of this molecule. CS proteins bind to heparam sulfate proteoglycans (HSPGs) from the basolateral cell surface of hepatocytes in the Disse space and that this interaction occurs between the CS protein's conserved I- and II-plus regions and heparin-like oligosaccharides and/or heparam sulfate[21].

For resonant consensus spectra estimation, the following CS sequences were included into the analysis:

1. Circumsporozoite protein Plasmodium berghei
2. Circumsporozoite protein Plasmodium yoeliiyoelii
3. Circumsporozoite protein Plasmodium brasilianum
4. Circumsporozoite protein Plasmodium knowlesi (strain H)
5. Circumsporozoite protein Plasmodium vivax (strain Belem)
6. Circumsporozoite protein Plasmodium berghei (strain anka)
7. Circumsporozoite protein Plasmodium reichenowi
8. Circumsporozoite protein Plasmodium falciparum (isolate Wellcome)
9. Circumsporozoite protein Plasmodium simium
10. Circumsporozoite protein Plasmodium cynomolgi (strain Berok)
11. Circumsporozoite protein Plasmodium falciparum (isolate Thailand)
12. Circumsporozoite protein Plasmodium cynomolgi (strain Ceylon)
13. Circumsporozoite protein Plasmodium cynomolgi (strain London)
14. Circumsporozoite protein Plasmodium cynomolgi (strain Mulligan/NIH)
15. Circumsporozoite protein Plasmodium cynomolgi (strain Gombak)
16. Circumsporozoite protein Plasmodium maarae
17. Circumsporozoite protein Plasmodium vivax (strain SalvadorI)
18. Circumsporozoite protein Plasmodium falciparum (isolatele 5)

Apical Membrane Antigen 1 (AMA-1)

The AMA-1 is expressed late in the erythrocytic replication cycle, the 83-kDa membrane protein is initially found in apical micronemes of the merozoite. The AMA-1 is subsequently processed to a 66-kDa form by removal of an N-terminal prosequence and is translocated to the merozoite surface, where additional proteolytic cleavage occurs. AMA-1 has role in orienting the merozoite during invasion of erythrocytes. It is also expressed during the sporozoite and heptic stages. Anti-AMA-1 antibodies tend to be present among individuals with acquired natural immunity to malaria. On the other hand, repeated natural exposure often leads to high titres of IgG to AMA-1. Some authors consider that AMA-1 could become one of the most promising blood-stage malaria vaccine candidates[22]. However, the allelic polymorphism and other immunological escape mechanisms could loom as major obstacles to this goal. The AMA-1 has also being described as a target for hepatocyte invasion by sporozoites, suggesting that AMA-1 could be considered as a potential candidate to be included in a multi-stage malaria vaccine, targeting both erythrocytic and pre-erythrocytic stages[23].

For resonant consensus spectra estimation, the following AMA-1 sequences were included into the analysis:

1. Apical membrane antigen 1 AMA-1 Plasmodium falciparum (isolate 3D7)
2. Apical membrane antigen 1 AMA-1 Plasmodium chabaudi
3. Apical membrane antigen 1 AMA-1 PF83 Plasmodium falciparum (isolate FC27 / Papua New Guinea)
4. Apical membrane antigen 1 AMA-1 AG352 Plasmodium fragile

5. Apical membrane protein-1 AMA-1 Imported Plasmodium vivax Imported

6. Apical merozoite antigen 1 AMA1 Plasmodium knowlesi

EBA175

The EBA175 protein is a blood stage antigen that aids binding of the merozoite to host erythrocytes[24]. The RII region of the protein is highly conserved among the various strains of *P. falciparum*. Sera from volunteers had anti-EBA175 RII antibodies that demonstrated modest parasitic growth inhibition and inhibition of parasitic binding to erythrocytes in vitro. The EBA175 binds to sialic acid and glycophorin A during RBC invasion[24]. The EBA 175 also binds to RBC Band 3 protein, thus participating in at least two major mechanisms of RBC invasion[26-28].

For resonant consensus spectra estimation, the following EBA 175 sequences were included into the analysis:

1. Erythrocyte binding antigen EBA Plasmodium falciparum (isolate Camp/Malaysia)

2. Erythrocyte binding antigen 175 eba-175 MALP. Plasmodium falciparum (isolate D)

3. Erythrocyte invasion ligand JSEBL/EBA-175 Plasmodium reichenowi

4. Erythrocyte binding antigen EBA-175 Plasmodium sp. Gorilla clade G

5. Erythrocyte binding antigen EBA-175 Plasmodium sp. Chimpanzee clade C

6. Erythrocyte binding antigen 175 EBA PRCDC Plasmodium reichenowi

7. Erythrocyte binding antigen Plasmodium falciparum SantaLucia

8. Erythrocyte binding antigen Plasmodium falciparum (isolate NF)

PfEMP1 (Plasmodium falciparum erythrocyte membrane protein)

The PfEMP1 has been identified as the rosetting ligand of Plasmodium[29]. Rosetting is the adhesion of infected erythrocytes with uninfected erythrocytes in the vasculature of the infected organ, and is associated with severe malaria[30,31]. The PfEMP-1 proteins were found to bind to a wide range of endothelial receptors, such as CD36, intercellular adhesion molecule 1 (ICAM-1), chrondroitin sulfate A (CSA), platelet endothelial cell adhesion molecule (PECAM), vascular cell adhesion molecule (VCAM), hyaluronic acid, heparam sulfate, and other molecules, such as complement receptor 1, immunoglobulins G and ABO blood group antigens[12].

For consensus resonant spectra estimation, the following PfEMP1 sequences were included into the analysis:

1. PfEMP1FCR3-C6var1 PfEMP1FCR3-C5var1 Plasmodium falciparum

2. PfEMP1MCK-var1 Plasmodium falciparum

3. PfEMP1FVOvar1 Plasmodium falciparum

4. Erythrocyte membrane protein1 variant 3 Imported PfEMP1protein

5. PfEMP1DBLa Plasmodium falciparum

6. PfEMP1 P154 var1 Plasmodium falciparum

7. PfEMP1 P154 var2 Plasmodium falciparum

8. PfEMP1 P154 var3 Plasmodium falciparum

9. PfEMP1 PfEMP1PAvar11 Plasmodium falciparum

10. PfEMP1Dd2var7 Plasmodium falciparum

11. PfEMP1 CS294var3 Plasmodium falciparum

12. PfEMP1 P132var1 Plasmodium falciparum

13. PfEMP1 R29R-var5 Plasmodium falciparum

Signal Peptide protease (PfSPP)

The PfSPP binds to band 3 proteins from RBC membrane. It has been regarded as a potential target of anti-malarial drug development(28,32).

For resonant consensus spectra estimation, the following SPP sequences were included into the analysis:

1. Signal peptide peptidase SPP Plasmodium falciparum

2. Signal peptide peptidase SPP Plasmodium reichenowi

3. Signal peptide peptidase PKH_124910 Plasmodium knowlesi (strain H)

4. Signal peptide peptidase-like 2A SPPL2A Homo sapiens (Human)

RBC Band 3 protein

For studying putative interactions, RBC Band3[28] protein was also analyzed. Band 3 protein is a ubiquitous membrane transport protein found in the plasma membrane of diverse cell types and tissues. It is the major integral transmembrane protein of the erythrocyte plasma membrane, comprising 25% of the total membrane protein. It exists as a dimer and performs the important function of allowing the efficient transport of bicarbonate across erythrocyte cell membranes in exchange for chloride ion, as well as lactate influx to RBC. It has been hypothesized that the main route of RBC invasion by merozoites occurs through a Band 3 dependent pathway[28]. The presence of Band-3-like protein in hepatocytes has been suggested as well[33].

For resonant consensus spectra estimation, the following Band 3 sequences were included into the analysis:

1. Band 3 anion transport protein Slc4a1 Mus musculus (Mouse)

2. Band 3 anion transport protein SLC4A1 Homo sapiens (Human)

3. Band 3 anion transport protein Slc4a1 Rattus norvegicus (Rat)

4. Solute carrier family 4, anion exchanger, member 2 (Erythrocyte membrane protein band 3-like 1) SLC4A2 Pan troglodytes (Chimpanzee)

RESULTS

Determination of consensus spectra

In figures 1a, 1b and 1c consensus spectra for different groups of sequences are provided. As it can be seen from figure 1, the 18 CS frequencies exhibited a prominent frequency peak at f=0.0020. This frequency is shared also by EBA sequences as shown in figure 2, as well as PfEMP sequences as shown in figure 3. CS from plasmodium species with dormant stages (*P. cynomolgi* and *P. vivax*) exhibited an additional frequency peak at f=0.3340 as shown in figure 1b. By contrast, CS from *P. falciparum* only presented the common frequency peak at f=0.0020 as shown in figure 1c.

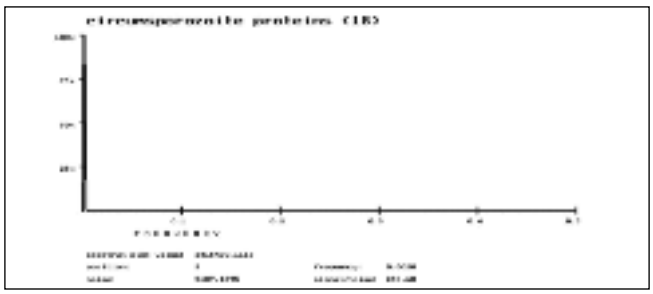

***Figure 1a**. Consensus spectrum for the eighteen sequences of Circumsporozoite protein analyzed. The prominent frequency peak at f=0.0020 is apparent.*

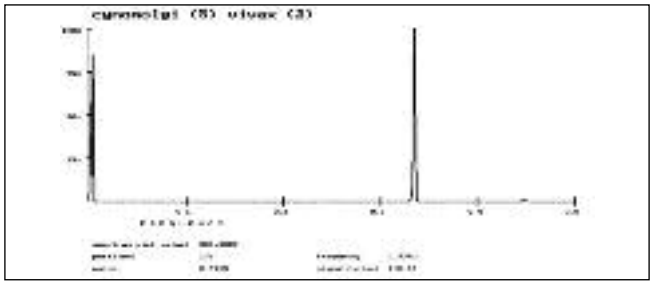

***Figure 1b**. Consensus spectrum for the seven sequences of Circumsporozoite protein from Plasmodium cynomolgi and Plasmodium vivax. Together with the frequency peak at f=0.0020, a new frequency peak appears at f=0.3340.*

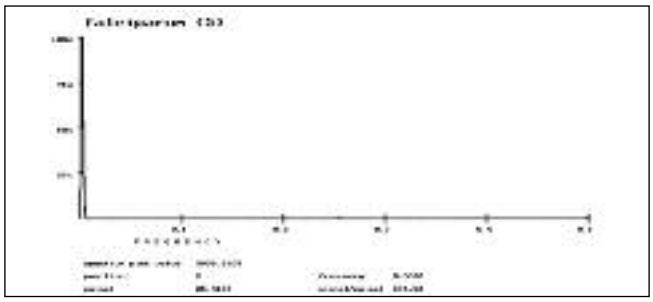

***Figure 1c**. Consensus spectrum for the five sequences of Circumsporozoite protein from Plasmodium falciparum. Only the frequency peak at f=0.0020 is apparent.*

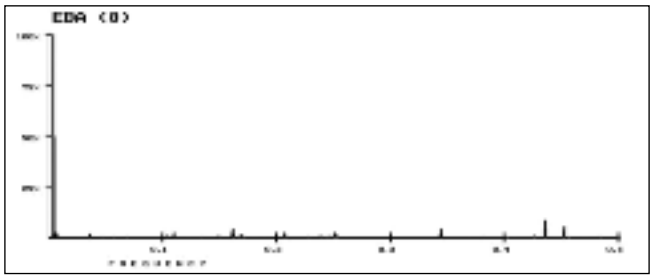

***Figure 2**. Consensus spectrum for the eight sequences of EBA protein have been studied. One frequency peak at f=0.0020 is apparent.*

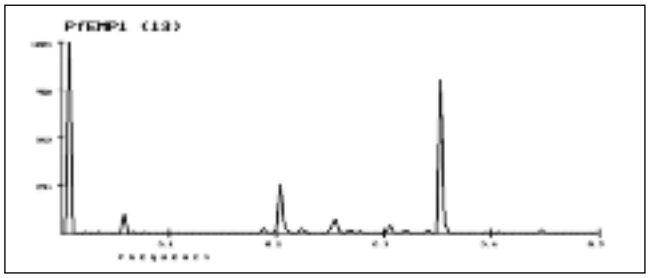

***Figure 3**. Consensus spectrum for the thirteen sequences of PfEMP1 protein studied. Frequency peaks at f=0.0020, as well as at f=0.3300 are apparent.*

Thus, all analyzed malaria proteins, including circumsporozoite proteins, PfEMP1, erythrocyte binding antigen EBA, have shown one characteristic and common frequency at range of f=0.0020-0.0078 within the calculation error. Such common frequency is raising the possibility that this frequency is vital for malaria.

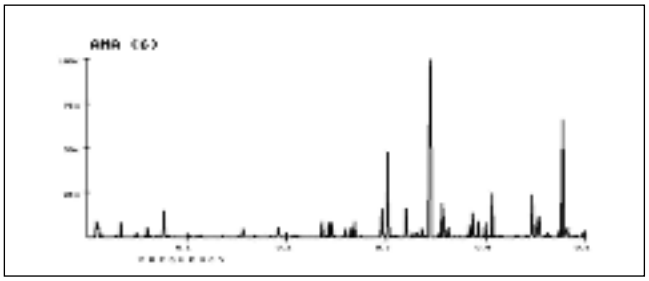

***Figure 4.** Consensus spectrum for the six sequences of AMA protein studied. The striking difference with spectra from figures 1-3 is apparent. AMA consensus spectrum does not have any prominent characteristic. The main common frequency is at f=0.3438. This frequency is close to second important frequency in PfEMp1 and to CS in non falciparum parasites.*

Plasmodium protein binding to RBC Band 3 protein

From comparing figure 5 with figure 6, evidence for an interaction between the malaria protein and the RBC protein frequency at f=0.1100 is apparent. The requirement for interaction as per the RRM is that interacting proteins have the same common characteristic frequency as well as opposite phase difference close to 3.14rad or 180o at this frequency for the pair of interacting proteins. Phase at frequency of f=0.1100 for RBC Band 3 protein is found to be at -2.38rad while phase at the same frequency for EBA was found to be at +0.38rad making the phase difference between these two proteins to be 2.76rad which can be considered to be close to 3.14rad as presented in phase circles in figure 7. Thus, the RRM revealed interaction between erythrocyte binding antigen (EBA) and Erythrocyte membrane protein band 4.2 at the common characteristic frequency of f=0.1100.

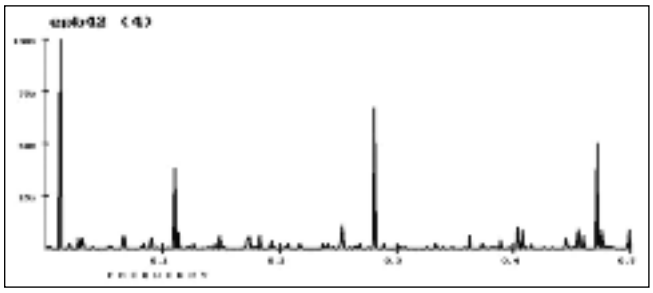

***Figure 5.** Consensus spectrum for the four RBC Band 3 (epb42)proteins analyzed.*

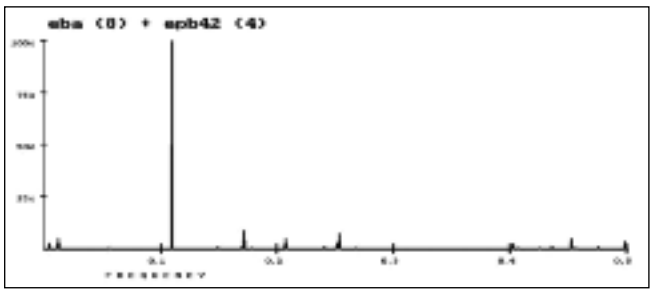

***Figure 6.** Evidence for a common frequency at f=0.1100. Eight EBA and four RBC Band 3 proteins have been studied.*

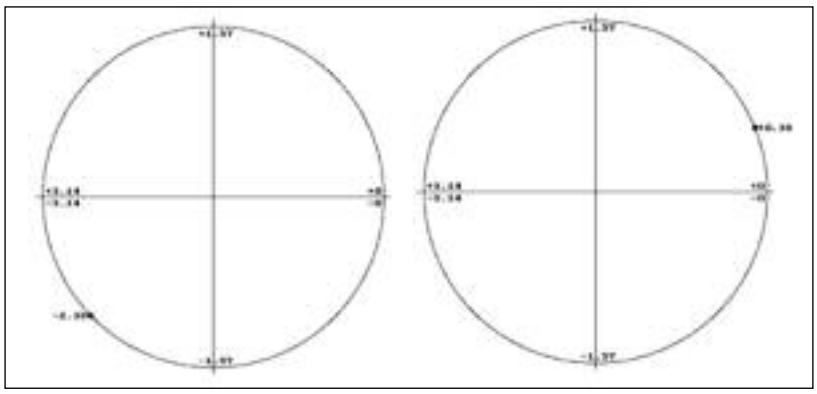

Figure 7. *Phase estimation, presented on a phase circle, for RBC Band 3 protein (left) and EBA (Right) for frequency at f=0.1100. It can be seen from the phase circle that the phase difference is close to be opposite.*

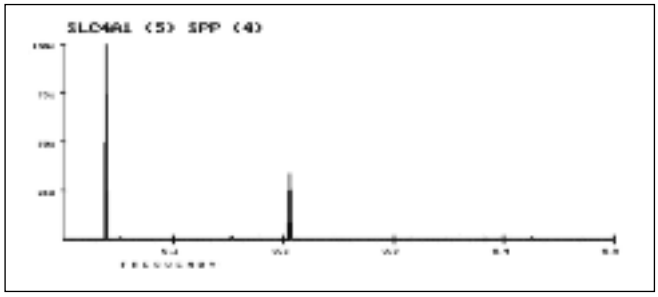

Figure 8. *Common frequency at f=0.0390 between RBC Band 3 and PfSPP.*

A significant common frequency between SPP and SLC4A1 human was found to be at f=0.0390 with opposite phases at this frequency: phase for slc4 is at +2.94rad and for SPP at +0.16rad making the phase difference of 2.78rad to be close enough to 3.14rad (opposite phase) as it can be seen from figure 9. This result thus, according to RRM, revealed interaction between SLCA4 human protein and PfSPP at frequency of f=0.0390.

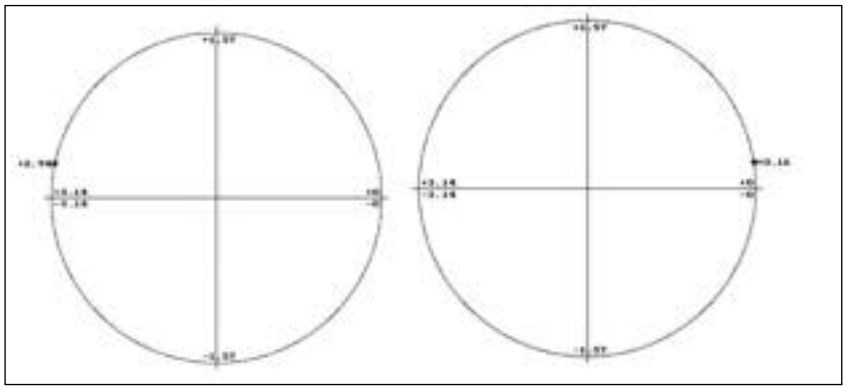

Figure 9. *Phase estimation for SLCA4 human protein (left) and PfSPP (right) for frequency at f=0.0390. It can be seen from the phase circle that the phase difference is close to be opposite.*

DISCUSSION

The application of a new approach to the study of a long standing complex subject, in our opinion, should start with evidences pointing to plausibility, on the basis of independent 'prediction' of well-established facts. If this condition is met, then the method seems reliable for predicting new, hitherto undiscovered facts. Available literature suggests that in the case of malaria parasite invasion, very little is known and a big extent of facts is to be discovered. In this case, the RRM, with its straight forward answers to a big repertoire of questions about protein-to-protein interactions seems to be nicely tailored for state-of-the-art malaria research.

From the results of our study, the following ones corroborate previously known facts from literature:

• RBC Band 3 protein attaches to both PfEMP and EBA, apparently through different mechanisms

• CS from different Plasmodium species do share similar biological function

• PfEMP1 from different Plasmodium species do share similar biological function

• EBA from different Plasmodium species do share similar biological function

Rather unexpected, but still explainable on the light of published data, were the following results.

Two frequencies emerged as being common to several proteins: frequency at f=0.0020 is shared by CS, EBA, and PfEMP1, where frequency at f=0.3400 is common to AMA, CS from non-*falciparum Plasmodia* as well as PfEMP1. We hypothesize that these two frequencies correspond to two distinct and relevant aspects of Plasmodium invasion. It is interesting to notice that the frequency at f=0.0020 is shared by proteins involved in both hepatocyte and RBC stages of invasion. Probably frequency at f=0.0020 is associated to membrane attachment processes, either to hepatocyte or RBC membrane. This idea finds support in the fact that heparam sulfate binds to CS(21), EBA[34] and PfEMP1[35]. This might suggest that frequency at f=0.0020 reflects cell recognition through glycans. On the other hand hepatocytes and RBC share several common integral membrane proteins, being Band 3 one of them.

It is known that AMA acts from inside the RBC, contributing to the re-orientation of penetrated merozoites[36]. This added to evidences for AMA playing a role during hepatocyte invasion, where it is sequestered into the hepatocyte cytoplasm could suggest that frequency at f=0.3400 is associated to intracellular invasion mechanisms, both hepatocyte and RBC. This is also in agreement with evidences about expression of CS in intracellular hypnozoites from Plasmodium vivax[37], however, this needs to be confirmed.

Although these results look unexpected in some way and although malaria parasite has very complex life cycle and set of interactions, these results, according to RRM postulate, are showing that there are only two distinct characteristics of the Plasmodium interaction with RBC and hepatocyte. In other words, it seems that such a complex process of Plasmodium invasion of RBC can be simplified with only two RRM characteristic frequencies relevant for this

process. Knowing these characteristic frequencies, it is possible to interfere with the process by either designing proteins that will directly interfere with the process and thus represent the medication or by designing the proteins that will act as vaccine inducing antibodies which will be able to recognize Plasmodium. This approach has been already used in a number of successful examples.

In the case of Fibroblast Growth Factors (FGF) two characteristic frequencies were identified: one related to receptor recognition and another related to „growth activity". The aim of that particular project was to design peptide which can competitively bind to the FGF receptor but without inducing growth. Using only receptor recognition frequency the 16-mer peptide was designed and experimentally tested. It indeed had receptor recognition activity without inducing growth[14-16,38].

A similar idea was used to mimic myxoma virus oncolytic function. Myxoma virus (MV) is a rabbit-specific poxvirus pathogen that also exhibits a unique tropism for human tumor cells and is dramatically oncolytic for human cancer xenografts. The RRM characteristic frequency for MV proteins was identified and used to design peptides that were experimentally shown to mimic myxoma virus oncolytic function[39,40].

The most relevant example is in the case of HIV virus, where the one common RRM frequency was identified for all HIV envelope protein despite their high variability. This frequency was used to design peptide that can immunologically mimic all HIV isolates and thus could be a good candidate for vaccine[14-16,41].

Among the difficulties for developing a new antimalarial vaccine is the repertoire of immune escape mechanisms displayed by *Plasmodium* molecules. It is to be expected, similarly as in the case of HIV virus, that RRM-designed molecules based on characteristic common to most relevant Plasmodium proteins, will be robust to polymorphisms, one of the main obstacles for a malaria vaccine creation.

Thus application of the RRM to the study of malaria proteins corroborated some previously known data and predicted new ones, providing new insights to our understanding of one of the major public health challenges of our time.

Once again, it has been shown that the RRM approach, despite protein variability, is capable to identify general interaction relevant characteristics and thus could enable design of drugs and vaccines that can be more general and robust.

Acknowledgment

We would like to acknowledge the support from AMALNA Consulting for this research.

Sažetak

Invazija ljudskog organizma plazmodijum parazitom uključuje kompleksne interakcije gde glavnu ulogu igraju zajedno ljudski i plazmodijum proteini. U ovom radu smo analizirali proteine koji igraju glavnu ulogu u toj invaziji uz pomoć metode analize signala, model rezonantnog prepoznavanja (RRM). RRM je fizičko-matematički prilaz, koji analizira proteinske interakcije i baziran je na rezonantnom prepoznavanju između karakterističnih frekvencija u distribuciji energija slobodnih elektrona u proteinima. Glavne karakteristike u procesu invazije mogu da se sumiraju uz pomoc određenih rezonantnih frekvencija zajedničkih za grupe proteina koji učestvuju u plazmodium infekciji. Rezultati ovog rada potvrđuju rezultate iz postojeće literature: interakcija band 3 proteina iz Red Blood Cell sa Erythrocyte Binding Antigen (EBA) i Signal Peptide Protease (SPP), sa različčitim mehanizmom zajedničkim za Circumsporozoite protein, EBA, SPP i PfEMP. Identifikovane su dve karakteristične frekvencije važne za proces invazije parazita: za frekvenciju f=0.0200 se pretpostavlja da je vezana za prepoznavanje ćelijske membrane domaćina, dok frekvencija f=0.3400 verovatno predstavlja unutar ćelijske interakcije merozita i hipnozita. Ovi rezultati predstavljaju važan uvid u interakcije između proteina za vreme infekcije plazmodijumom i mogu se dalje upotrebiti za dizajniranje novih sintetičkih peptida, koji mogu da posluže kao kandidati za vakcine ili lekove za malariju.

REFERENCES

1. Patel SS, King CL, Mgone CS, Kazura JW, Zimmerman PA: Gerbich antigen blood group and band 3 polymorphisms in two malaria holoendemic regions of Papua New Guinea, *American Journal of Hematology*, 2004; 75, pp. 1–5.

2. Foo LC, Rekhraj V, Chiang GL, Mak JW: Ovalocytosis protects against severe malaria parasitemia in the Malayan aborigines, *Am J Trop Med Hyg*, 1992; 47, pp. 271-2755.

3. Urgell RA, Lin E, Manning L, Rarau P, Laman M, Senn N, Grimberg BT, Tavul L, Stanisic DI, Robinson LJ, Aponte JJ, Dabod E, Reeder JC, Siba P, Zimmerman PA, Davis TME, King CL, Michon P, Mueller I.: Reduced risk of Plasmodium vivax malaria in Papua New Guinean children with Southeast Asian ovalocytosis in two cohorts and a case-control study, *PLoS Medicine,* 2012; 9, e1001305.

4. Ayi K, Min-Oo G, Serghides L, Crockett M, Kirby-Allen M Quirt I, Gros P, Kain KC: Pyruvate kinase deficiency and malaria, *N Engl J Med*, 2008; 358, pp. 1805-1810.

5. Chotivanich K, Udomsangpetch R, Pattanapanyasat K, Chierakul W, Simpson J, Looareesuwan S, White N. Hemoglobin E.: A balanced polymorphism protective against high parasitemias and thus severe P falciparum malaria, Blood, 2002; 100, pp. 1172-1176.

6. Eridani S. Sickle cell protection from malaria, *Hematol Rep*, 2011; 3, e24.

7. Williams TN, Mwangi TW, Wambua S, Peto TEA, Weatherall DJ, Gupta S, Recker M, Penman BS, Uyoga S, Macharia A, Mwacharo JK, Snow RW, Marsh K: Negative epistasis between the malaria-rotective effects of α+-tha-

lassemia and the sickle cell trait, Nat Genet, 2005; 37, pp: 1253–1257.

8. Cui L, Su XZ: Discovery, mechanisms of action and combination therapy of artemesinin, *Expert Rev Anti Infect Ther,* 2009; 7, pp: 999-1013.

9. Singh SK, Hora R, Belrhali H, Chitnis CE, Sharma A: Structural basis for Duffy recognition by the malaria parasite Duffy-binding-like domain, *Nature*, 2006; 439, pp: 741-744.

10. Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, Guthmann JP, Nosten F, Carlton J, Looareesuwan S, Nair S, Sudimack D, Day NPJ, Anderson TJC, White, NJ: Relapses of *Plasmodium vivax* infection usually result from activation of heterologous hypnozoites, *Journal of Infectious Diseases*, 2007; 195, pp: 927-933.

11. Moorthy VS, Newman RD, Okwo-Bele JM: Malaria vaccine technology roadmap, *The Lancet*, 2013; 382, pp: 1700-1701.

12. Ferreira MU, da Silva Nunes M, Wunderlich G: Antigenic diversity and immune evasion by malaria parasites, *Clin. Diagn. Lab. Immunol*., 2004; 1, pp: 987-995.

13. Veiga MI, Ferreira PE, Jörnhagen L, Malmberg M, Kone A, Schmidt BA, Petzold M, Björkman A, Nosten F, Gil JP: Novel Polymorphisms in Plasmodium falciparum ABC Transporter Genes Are Associated with Major ACT Antimalarial Drug Resistance, *PLoS ONE*, 2011; 6, e20212.

14. Cosic I: Macromolecular bioactivity: is it resonant interaction between macromolecules?-Theory and applications, *IEEE Trans. on Biomedical Engineering,* 1994; 41, pp: 1101-1114.

15. Cosic I: Virtual spectroscopy for fun and profit, *Biotechnology,* 1995; 13, pp: 236-238.

16. Cosic I: The Resonant Recognition Model of Macromolecular Bioactivity: Theory and Applications. *Birkhauser Verlag*, Basel, 1997.

17. Hernandez Caceres JL, Cosic I, Cosic D: Retroviral proteases viewed through the resonant recognition model, *MD-Medical Data*, 2014; 6(2), pp: 117-123.

18. Cosic I, Cosic D: Balancing insulin stability and activity – Rational approach, *MD-Medical Data*, 2014; 6(1), pp: 7-10.

19. Ying P, Shakibaei M, MS Patankar, Clavijo P, Beavis RC, GF, Frevert U: The malaria circumsporozoite protein: interaction of the conserved regions i and ii-plus with heparin-like oligosaccharides in heparan sulfate, *Experimental Parasitology*, 1997; 85, pp: 168–182.

20. Stewart VA, McGrath SM, Dubois PM, Pau MG, Mettens P, Shott J, Heppner DG: Priming with an adenovirus 35-circumsporozoite protein (CS) vaccine followed by RTS,S/AS01B boosting significantly improves immunogenicity to Plasmodium falciparum CS compared to that with either malaria vaccine alone, *Infect Immune,* 2007; 75, pp: 2283–2290.

21. Cerami C, Frevert U, Sinnis P, Takacs B, Nussenzweig V: Rapid Clearance of Malaria Circumsporozoite Protein (CS) by Hepatocytes, *J. Exp. Med,* 1994; 179, pp: 695-701.

22. Yandar N, Pastorin G, Prato M, Bianco A, Patarroyo ME, Lozano JM: Immunological profile of Plasmodium vivax AMA-1 N-terminus peptide-carbon nanotube conjugate in an infected *Plasmodium berghei* mouse model, *Vaccine*, 2008; 26, pp: 5864-5873.

23. Silvie O, Franetich JF, Charrin S, Mueller MS, Siau A, Bodescot M, Rubinstein E, Hannoun L, Charoenvit Y, Kocken CH, Thomas AW, van Gemert GJ, Sauerwein RW, Blackman MJ, Anders RF, Pluschke G, Mazier D: A Role for Apical Membrane Antigen 1 during Invasion of Hepatocytes by Plasmodium falciparum Sporozoites, *The Journal of Biological Chemistry,* 2004; 279, pp: 9490-9496.

24. Rodriguez LE, Urquiza M, Ocampo M, Suarez J, Curtidor H, Guzman F, Vargas LE, Triviños M, Rosas M Patarroyo ME: *Plasmodium falciparum* EBA-175 kDa protein peptides which bind to human red blood cells, *Parasitology,* 2000; 120, pp: 225-235.

25. Cowman AF, Crabb BS: Invasion of red blood cells by malaria parasites, Cell, 2006; 124, pp: 755–766.

26. Mayer DG, Kaneko O, Hudson-Taylor DE, Reid ME, Miller LH: Characterization of a *Plasmodium falciparum* erithrocyte-binding protein paralogous to EBA-175, *PNAS,* 2001; 98, pp: 5222–5227.

27. Wanaguru M, Crosnier C, Johnson S, Rayner JC, Wright GJ: Biochemical analysis of the *Plasmodium falciparum* Erythrocyte-binding Antigen-175 (EBA175)-Glycophorin-A interaction. Implications for vaccine design, *The Journal of Biological Chemistry*, 2013; 288, pp: 32106-32117.

28. Li X, Chen H, Oh SS, Chishti AH: A Presenilin-like protease associated with Plasmodium falciparum micronemes is involved in erythrocyte invasion, *Molecular and Biochemical Parasitology,* 2008; 158, pp: 22–31.

29. Chen Q, Barragan A, Fernandez V, Sundström A, Schlichtherle M, Sahlén A, Carlson J, Datta S, Wahlgren M: Identification of *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) as the rosetting ligand of the malaria parasite P. falciparum, *The Journal of Experimental Medicine*, 1998; 187, pp: 15-23.

30. Rowe JA, Moulds JM, Newbold CI, Miller LH: *P. falciparum* rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1, *Nature*, 1997; 388, pp: 292-295.

31. Kriek N, Tilley L, Horrocks P, Pinches R, Elford BC, Ferguson DJ, Lingelbach K, Newbold CI: Characterization of the pathway for transport of the cytoadherence-mediating protein, PfEMP1, to the host cell surface in malaria parasite-infected erythrocytes, *Molecular microbiology*, 2003; 50, pp: 1215-1227.

32. Goel VK, Li X, Chen H, Liu SC, Chishti AH, Oh SS: Band 3 is a host receptor binding merozoite surface protein 1 during the Plasmodium falciparum invasion of erythrocytes, *PNAS*, 2003; 100, pp: 5164–5169.

33. Kay MB, Tracey CM, Goodman JR, Cne JC, Bassel PB: Polypeptydes immunologically related to band 3 are present in nucleated somatic cells, *Proc. Natl. Acad. Sci.* USA, 1983; 80, pp: 6882-6886.

34. Sinnis P, Sim BKL: Cell invasion by the vertebrate stages of Plasmodium, *Trends in microbiology*, 1997; 5, pp: 52-58.

35. Voght AM, Barragan A, Chen Q, Kironde F, Spillmann D, Wahlgren M: Heparan sulfate on endothelial cells mediates the binding of Plasmodium falciparum-infected erythrocytes via the DBL1alpha domain of PfEMP1, *Blood*, 2003; 101, pp: 2405-2411.

36. Mitchell GH, Thomas AW, Margos G, Dluzewski AR, Bannister LH: Apical membrane antigen 1, a major malaria vaccine candidate, mediates the close attachment of invasive merozoites to host red blood cells, Infection and immunity, 2004; 72, pp: 154-158.

37. Bilsborough J, Carlisle M, Good MF: Identification of Caucasian CD4 T cell epitopes on the circumsporozoite protein of Plasmodium vivax. T cell memory, *The Journal of Immunology*, 1993; 15, pp: 890-899.

38. Cosic I, Drummond AE, Underwood JR, Hearn MTW: A New Approach to Growth Factor Analogue Design: Modeling of FGF Analogues, *Molecular and Cellular Biochemistry,* 1994; 130, pp: 1-9.

39. Cosic I, Pirogova E: Bioactive Peptide Design using the Resonant Recognition Model, *Nonlinear Biomedical Physics*, 2007; 1, pp: 1-7.

40. Istivan T, Pirogova E, Gan E, Almansour NM, Coloe PJ, Cosic I: Biological effects of a de novo designed myxoma virus peptide analogue: Evaluation of cytotoxicity on tumor cells, *PLoS ONE*, 2011; 6, e24809.

41. Krsmanovic V, Biquard JM, Sikorska-Walker M, Cosic I, Desgranges C, Trabaud MA, Whitfield JF, Durkin JP, Achour A, Hearn MT: Investigation into the cross-reactivity of rabbit antibodies raised against nonhomologous pairs of synthetic peptides derived from HIV-1 gp120 proteins, *J. Peptide Res,* 1998; 52, pp: 410-412.

42. Cosic I, Lazar K, Cosic D: Cellular Ageing – Telomere, Telomerase and Progerin Analysed using Resonant Recognition Model, *MD-Medical Data*, 2014; 6(3), pp: 205-209.

■ The invited paper was received on 02.12.2014.