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Possibility to interfere with malaria parasite activity using specific electromagnetic frequencies

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Abstract

The absence of clear breakthrough in malaria combat could support the need for different ways of tackling the disease that are substantiated by conceptually new bases. The main idea of this research is to analyze possibility to interfere with malaria parasite activity using specific resonant electromagnetic frequencies. Although the idea to combat malaria infection with electromagnetic frequencies is not new, we will here present unique approach, so called Resonant Recognition Model (RRM) to specifically identify electromagnetic frequencies mostly important for interference with malaria infection. The RRM is calculating periodicities (frequencies) in distribution of free electron energies along protein sequence which are relevant for protein function/interaction. When charge transfer through protein backbone is considered then it can produce electromagnetic radiation of specific frequency depending on charge velocity. Ten groups of proteins relevant for *Plasmodium* interactions were analyzed.

Each of ten groups of proteins have at least one significant characteristic frequency peak at one of the following RRM frequencies: $f = 0.002$, $f = 0.11$ or $f = 0.34$. This suggests that the diversity of proteins participating in *Plasmodium* invasion could be represented with only three RRM frequencies. Depending on the charge transfer mechanism (velocity) along the protein, different electromagnetic resonant frequencies are expected. Based on presented results, we suggest that the RRM frequency of $f = 0.002$ (related to 2-5THz), to be regarded as crucial for *Plasmodium* infectivity and possibly for interfering with invasion process. Although this far infrared electromagnetic frequency cannot penetrate human body more than down to 4 cm, such radiation can be of great help in combating *Plasmodium*, since a sizeable part of parasite remain in the skin for hours after the mosquito bite. In addition the specific RRM frequency is capable to resonantly initiate a whole cascade of protein-protein (DNA, RNA) interactions directed to the specific biological activity which could contra-act *Plasmodium* infection.

Keywords: Resonant Recognition Model, Malaria, Plasmodium, Macromolecular interaction, Electromagnetic radiation

Background

This research is devoted to the study of malaria, one of the most challenging global health concerns of our time. In humans, malaria is caused by several species of the parasite *Plasmodium* (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium knowlesi*). During its lifecycle, *Plasmodium* parasites either the human host or the *Anopheles* mosquito. For this, a sophisticated

set of redundant invasion mechanisms is used, although the puzzle of these mechanisms is still far from being solved. At proteomic level, there are *Plasmodium* proteins interacting with human host proteins, as well as *Plasmodium* proteins interacting with the *Anopheles* mosquito proteins [1–7].

The main idea of this research is to analyze the possibility to interfere with malaria parasite activity using specific resonant electromagnetic frequencies. Resonant recognition in a nonlinear process. The idea to combat malaria infection with electromagnetic frequencies is not new, as there has been an attempt to design system that can kill mosquitos using infrared electromagnetic radiation [8]. Here, we are presenting approach to specifically interfere with infection, rather than killing mosquitos, with specific characteristic electromagnetic frequencies calculated using unique, unconventional and innovative approach, so called Resonant Recognition Model (RRM). The RRM proposes that protein-protein, protein-DNA and protein-RNA interactions are based on electromagnetic resonant recognition with distinctive characteristic frequency for each interaction [9–11].

The RRM approach is based on physical–mathematical description that has found growing support along the last 30 years [12–16]. According to the RRM, we propose that protein recognition during parasite invasion takes place through exchange of electromagnetic radiation at very specific frequencies. We use this unique approach to identify the specific frequencies of interaction between *Plasmodium* and both human and *Anopheles* mosquito cells with possibility to interfere with these frequencies pursuing to prevent malaria infection. Similar approach using the RRM has been proposed to combat Ebola infection [15].

In our previous research we have analyzed interaction between *Plasmodium* proteins and human proteins involved in malaria infection with the objective of proposing a promising RRM based vaccine candidate [17]. Here we build up on these results to find out the main specific frequencies of initial recognition between *Plasmodium* and human proteins, as well as *Anopheles* mosquito proteins. The new proteins analyzed were three *Plasmodium* proteins associated to mosquito invasion (CTRP, TRAP and Enolase), as well as one Anophelian protein involved in the immune response of the mosquito against *Plasmodium* invasion (ICHIT). We estimated RRM characteristic frequencies for groups of proteins involved in *Plasmodium* life cycle. Subsequently, the expected electromagnetic frequencies were estimated, assuming several plausible scenarios for charge movement mechanisms. Based on the obtained results, we propose that electromagnetic radiation at these frequencies will be able to interfere with the corresponding macromolecular interactions.

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 [9–11]. The RRM describes the most likely theoretical scenario for protein recognition and interaction managing to overcome the some drawbacks of traditional approaches based on structural complementarity. At the same time, the RRM includes a practical algorithm for detecting the resonant frequencies in a group of analogous/orthologous proteins. For that, a protein's primary structure is represented as a distribution

of free electron energies along the sequence with each amino acid in the sequence symbolized with the corresponding physical-chemical parameter describing free electron energies [9–11].

The resulting numerical series represents the distribution of the free electrons energies along the protein molecule. Such numerical series is then analyzed with digital signal analysis methods, using Fourier Transform, in order to extract information pertinent to the biological function. As the distance between amino acid residues in a polypeptide chain is 3.8 \AA , it can be assumed that the points in the numerical sequence are equidistant. For further numerical analysis, the distance between points in these numerical sequences is set at an arbitrary value of $d = 1$. Therefore, the maximum frequency in the spectrum is $F = 1/2d = 0.5$. The total number of points in the sequence influences the resolution of the spectrum only. Therefore, for an N -point sequence the resolution in the spectrum is equal to $1/N$. The n -th point in the spectral function corresponds to the frequency $f = n/N$.

In order to extract common spectral characteristics of sequences having the same or similar biological function, the multiple cross-spectral function is used. Comparing Fourier spectra of these energy distributions using multiple cross-spectral function (consensus spectrum), it has been found that proteins sharing the same biological function share the same periodicity (frequency) within energy distributions along the protein. This common frequency is proposed to be characteristic frequency for the biological function analyzed. In addition, it has been found that interacting macromolecules (protein/DNA/RNA) do share the same frequency, but exhibit opposite phase at this frequency [9–11, 17]. Thus, it has been proposed that the RRM frequencies characterize not only a general function, but also interaction between the particular protein and its target, which then can be considered to be resonant recognition.

Conceiving macromolecular interactions as resonant energy transfer between the interacting molecules, energy transfer can be foreseen as through oscillations of a physical field, possibly electromagnetic in nature. Since there is evidence that proteins and DNA/RNA have certain conducting or semi-conducting properties, charge moving through the macromolecular backbone and passing different energy stages caused by distribution of free electron energies along the protein sequence, can produce sufficient conditions for a specific electromagnetic radiation or absorption. The frequency range of this field depends on charge velocity. Initially, the RRM proposes that charge is travelling through macromolecular backbone in the form of solid state electrons at estimated velocity of $7.87 \times 10^5 \text{ m/s}$ [9–11]. For this velocity and the distance between amino acids in a protein molecule, which is 3.8 \AA , the frequency range for protein interactions was estimated to be in the range of 10^{12} Hz up to 10^{15} Hz (Cosic's scenario). These computational predictions were found to be related to biological function of the macromolecules by comparison with a number of experimental measurements [9, 10], as well as by direct experimental endorsement [13–16, 18].

With this scenario charge transfer is proposed to travel through protein backbone. However, if we take into account possibility for charge transfer through protein complex structures and in particular alpha helices, it could be in the form of solitons [19] (Davydov [20, 21], Hyman [22], Sinkala [23]), excitons (Davydov [20, 21], Sinkala [23], Yomosa [24], Pang [25]) and phonons (Yomosa [24], Pang [25], Ichinose [26]). These other forms of charge transfers proceed at different velocities ranging from 10^5 m/s for

solitons and some excitons all the way down to the speed of sound and small fractions of the speed of sound for phonons. Thus, with the same periodicities within proteins sequences, as determined by the RRM, different modalities of charge transfer can produce different resonant frequencies, which are not necessarily related to their protein biological function, but could be related to the protein and DNA/RNA resonances, in general. Such approach has been tested with tubulin and microtubule [27] and results have been experimentally proved [18]. In addition we have applied this approach to telomerase as example of proteins, telomere as example of DNA sequences, as well as TERT mRNA as an example of RNA macromolecules [28].

Sequences

We centered our study using RRM analysis on:

- proteins involved in parasite infection of red blood cells/hepatocytes,
- proteins involved in the Anopheline stage of *Plasmodium* life cycle,
- proteins proposed as vaccine candidates,
- human erythrocyte targets of *Plasmodium* invasion,
- proteins involved in mosquito stage of *Plasmodium* lifecycle.

For a full list of the eighty one analyzed sequences (allocated into ten groups of proteins), see Additional file 1. The following groups were included in current research:

Intestinal Chitin Binding Protein (ICHIT)

The ICHIT is a galectin with two putative chitin-binding domains separated by a polythreonine-rich mucin region. ICHIT's mucin domains participate in the formation of the extracellular matrix and in trapping microbial pathogens. The ICHIT is regarded as a marker of mosquito's immune response and is also the name of this protein's encoding gene, which is regarded as belonging to the 'Pattern Recognition Receptor' gene family [1]. Eight ICHIT sequences were analyzed.

Circumsporozoite and TRAP Related Protein (CTRP)

The CTRP is the first molecule reported to be essential for ookinete infectivity and consequently, mosquito transmission of malaria. It is produced in the mosquito-invasive, or ookinete stage and is a protein candidate for a role in ookinete adhesion and invasion of the mosquito midgut epithelium and is considered to be a transmission-blocking vaccine candidate. The CTRP contains two types of adhesive domains (von Willebrand factor type A-related and thrombospondin type 1-related) and has been detected at the site of contact with the basal lamina, suggesting that the junction so formed may induce the ookinete to transform into an oocyst, which has been supported by targeted disruption of CTRP experiments in vivo. The CTRP is also involved in ookinete locomotion into Anopheles midgut and has been shown to bind to laminin and collagen IV, major components of the basal lamina, prompting further suggestions of a putative role in the transformation of ookinete to oocyst. It was found that naturally induced humoral immunity against CTRP leads to transmission halt [3]. Six CTRP sequences were analyzed.

Thrombospondin-Related Anonymous Protein (TRAP)

The TRAP is a protein expressed in sporozoites. During anopheles invasion, the TRAP is a core player in sporozoite gliding, cell invasion and in vivo infectivity. It has been found that sporozoites carrying a mutated TRAP A-domain are impaired in salivary gland invasion, but not in gliding motility, suggesting that the two processes are functionally distinct and that the A-domain is involved in recognition and/or attachment to salivary gland receptor molecules. The TRAP is one of the few vaccine candidates proposed hitherto for *P. vivax*. *Plasmodium falciparum* TRAP contributes to the development of protection against severe malaria, suggesting that this molecule could increase the protective efficiency of available sporozoite malaria vaccines [2]. Seven TRAP sequences were analyzed.

Enolase

The *Plasmodium* Enolase is an essential enzyme in the glycolytic pathway catalyzing the conversion of 2-phosphoglycerate to phosphoenolpyruvate. Glycolytic enzymes seem to be promising candidates from this perspective, as energy production in *P. falciparum* depends entirely on the glycolytic pathway as the parasite and its mammalian host (erythrocytes) lack a complete Krebs cycle and active mitochondria. Interestingly, the Enolase also serves a separate function: it is exported to merozoite cell surface via a poorly understood mechanism. In *Plasmodium*, surface Enolase assists in the invasion of their host cells by binding plasminogen, a plasma protease precursor. It is an important target in mosquito's immune response, due to its nature as ookinete surface ligand. It apparently interacts with midgut epithelial cells. The Enolase has been considered as a promising vaccine candidate [5, 29]. Seven Enolase sequences were analyzed.

Circumsporozoite Protein (CS)

The CS plays a key role in hepatocyte invasion by *Plasmodium* sporozoites. This process proceeds apparently via interaction of CS with proteoglycans on the surface of the hepatocytes. The only phase-3 vaccine for malaria so far is based on CS [4, 30]. Eighteen CS sequences were analyzed.

Apical Membrane Antigen 1 (AMA-1)

The AMA-1 protein, is involved in merozoite reorientation, moving junction formation and parasitophorous vacuole resealing during erythrocyte invasion. It is also expressed during the sporozoite and hepatic stages. The AMA-1 has also being claimed as a target for hepatocyte invasion by sporozoites, suggesting that it could be considered as a potential candidate for a multi-stage malaria vaccine, targeting both erythrocytic and pre-erythrocytic human host stages [31, 32]. Six AMA-1 sequences were analyzed.

Erythrocyte Binding Antigen (EBA175)

The EBA175 protein is a blood stage antigen that aids binding of the merozoite to host erythrocytes. The EBA175 is regarded as an adhesin that binds to sialic acid and glycoporphin A during RBC invasion. The EBA175 also binds to RBC Band3 protein, thus participating in at least two major mechanisms of RBC invasion. Antibodies targeting EBA175 do inhibit RBC infection by *Plasmodium*. The EBA175 has been considered as a promising vaccine candidate [33]. Eight EBA175 sequences were the analyzed.

Plasmodium falciparum Erythrocyte Membrane Protein (PfEMP1)

The PfEMP1 has been described as the rosetting ligand of *Plasmodium*. Rosetting is the term used to describe the adhesion of infected erythrocytes with uninfected erythrocytes in the vasculature of the infected organ and is associated with severe malaria. The PfEMP1 proteins were found to bind to a wide range of endothelial receptors, such as CD36, intercellular adhesion molecule 1 (ICAM-1), chondroitin sulfate A, platelet endothelial cell adhesion molecule, vascular cell adhesion molecule, hyaluronic acid, heparan sulfate, and other molecules, such as complement receptor 1, immunoglobulins G and ABO blood group antigens. The PfEMP1 has been considered as a vaccine candidate [6]. Thirteen PfEMP1 sequences were analyzed.

Signal Peptide Protease (PfSPP)

The PfSPP binds to Band3 proteins from RBC membrane. It has been regarded as a potential target of anti-malarial drug development. Four PfSPP sequences were analyzed.

RBC Band3 protein

For studying putative interactions, the RBC Band3 protein was also analyzed. Band3 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 allows bicarbonate/chloride exchange across erythrocyte cell membranes, as well as lactate influx to RBC. It has been hypothesized that the main route of RBC invasion by merozoites occurs through a Band3 dependent pathway. The presence of Band3-like protein in hepatocytes has been suggested as well [7]. Four RBC Band3 sequences were analyzed.

Results and Discussion

Here, we used the RRM model to analyze the proteins involved in *Plasmodium* life cycle including interaction with human host. The aim was to find out characteristic frequencies for these functional groups of proteins and for their interactions. Subsequently, the aim was to identify electromagnetic frequencies related to these characteristic frequencies and related functions/ interactions. If we identify a frequency that is vital for *Plasmodium* interaction with the host then there is possibility to interfere with this frequency and prevent interaction with host (infection).

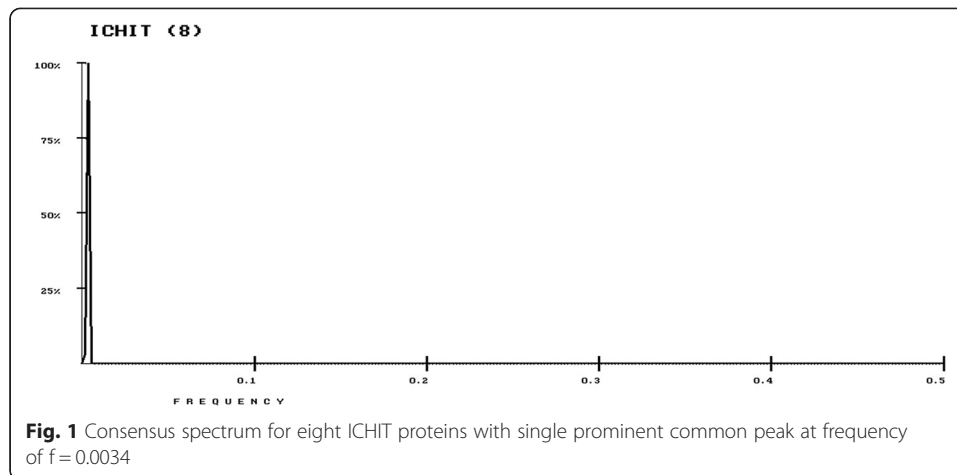
We have analyzed all listed groups of malaria related proteins and obtained the following results:

Anopheles proteins involved in Plasmodium invasion

Consensus spectrum for ICHIT is showing single prominent common peak at frequency of $f = 0.0034$, as presented in Fig. 1.

Plasmodium proteins involved in anopheles invasion

From the consensus spectrum for CTRP, as presented in Fig. 2, several peaks can be observed, perhaps reflecting the multiple functions ascribed to this protein. As apparent, the main peak appears at frequency of $f = 0.0186$. Other peaks appear at frequency of $f = 0.0034$, at frequency of $f = 0.11$ and at frequency of $f = 0.34$.



Consensus spectrum for TRAP is presented in Fig. 3. The main peak appears at frequency of $f = 0.2508$, whereas a second peak appears at frequency of $f = 0.11$.

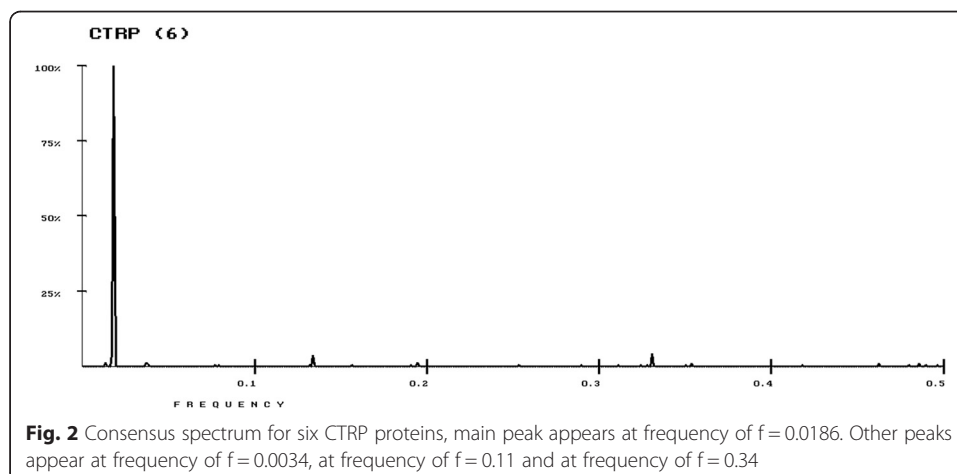
Consensus spectrum for Enolase is presented in Fig. 4. The main peak appears at frequency of $f = 0.3496$, whereas other peak appear at frequency of $f = 0.43$.

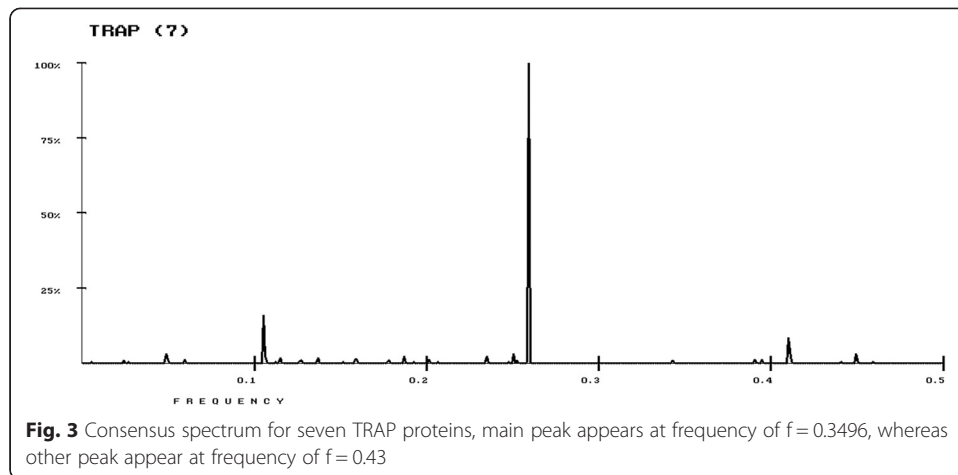
Plasmodium proteins involved in human host invasion

Consensus spectra for *Plasmodium* proteins involved in interaction with the human host have been discussed in detail in our previous paper [17]. Here we outline the most salient aspects of that study.

When the RRM was applied to eighteen CS proteins only one prominent peak appeared at frequency of $f = 0.002$. This frequency is shared also by EBA175 proteins, as well as PfEMP1 proteins. CS from *Plasmodium* species with dormant stages (*P. cynomolgi* and *P. vivax*) exhibited an additional peak at frequency of $f = 0.334$. However, CS from *P. falciparum* only presented the common peak at frequency of $f = 0.002$.

In summary, for *Plasmodium* proteins interacting with the human host, the frequencies of the main peaks are presented in Table 1.





Human proteins interacting with plasmodium proteins

The RBC Band3 proteins from red blood cells share the same frequency of $f = 0.11$ with EBA175, but with opposite phase at that frequency, thus confirming the known interaction between these two proteins during erythrocyte invasion [17].

Estimating electromagnetic wavelengths according to different charge transfer mechanisms

The RRM proposes interaction between macromolecules, including proteins, is based on resonant electromagnetic energy transfer. The frequency of this electromagnetic energy will depend on periodicity of free electron energy distribution along protein as calculated by the RRM, as well as upon the speed at which charges are traveling. Since different mechanisms of charge transfer have been proposed, more than one frequency will be associated to a single RRM frequency.

Different frequencies were identified, but the three following frequencies were found in the number of different groups of proteins and thus are of special interest: frequency of $f = 0.002$, frequency of $f = 0.11$ and frequency of $f = 0.34$. These three frequencies could be considered as the characteristic of parasite vitality in general. Thus, we propose that parasite vitality can be diminished by interfering with some of these three

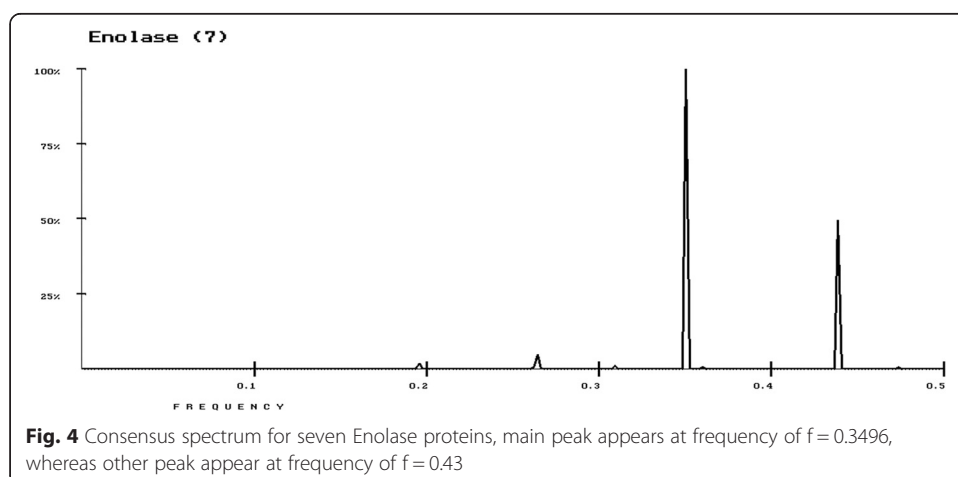


Table 1 Main peaks among *Plasmodium* proteins interacting with the human host

Protein	Peak I	Peak II
Circumsporozoite protein (All)	0.002	-
Circumsporozoite protein (species with dormant liver stage)	0.34	0.002
PfEMP1	0.002	0.34
AMA	0.34	0.475
SPP	0.039	0.22
EBA	0.002	-

RRM frequencies. To find out the corresponding electromagnetic frequencies we have introduced the different modalities of charge transfer, as described above. The corresponding electromagnetic frequencies depending on different charge velocities are presented in Table 2. These results have shown that possible resonant frequencies relevant to parasite vitality can be within different frequency ranges (THz, GHz, MHz and even KHz) depending on mechanism and charge velocity within the protein structure.

On the other hand the frequency at range of $f = 0.002-0.0078$ within the calculation error appears as a very prominent peak in four of the ten studied protein groups, including ICHIT, CS, EBA175 and PfEMP1. The roles played by these proteins are very diverse, however our results are showing that there is common characteristic frequency for all these proteins analyzed and thus we propose that the frequency of $f = 0.002$ is a key role player for *Plasmodium* vitality. In addition, we also narrow down to charge velocity, as proposed by Cosic's scenario, which has been shown in the past to be relevant for biological function of the proteins [27, 28]. Thus, we propose here that the most probable frequency to be used to interfere parasite activity is frequency of 2-5THz.

With this results in mind, it is possible to consider that instead of several hundreds of proteins, cell invasion mechanism can be assessed through a considerably fewer number of electromagnetic frequencies. Thus, RRM theory is proposing an alternative simpler way to handle the problem of malaria invasion.

Conclusion

Our results revealed that in spite of structural and functional diversity, as well as differences in their origin, ten studied protein groups shared at least one of the following three RRM frequencies ($f = 0.002$, $f = 0.11$ or $f = 0.34$). Particularly ubiquitous was frequency of $f = 0.002$ (0.002-0.0078), that appeared as the main peak of consensus spectra for proteins playing so diverse roles as pattern recognition receptor immunity in mosquitoes, hepatocyte invasion, antibodies to human proteins and erythrocytes' rosetting. Thus frequency of $f = 0.002$ has been proposed as the main RRM frequency involved in

Table 2 Electromagnetic Resonance Frequencies taking into consideration as proposed by different mechanisms of charge velocities for main malaria proteins characteristic frequencies

RRM Frequency	EMf THz per $v = 787000$ m/s (Cosic)	EMf THz per $v = 180000$ m/s (Yomosa)	EMf GHz per $v = 170$ m/s (Davydov)	EMf GHz per $v = 68$ m/s (Pang)	EMf MHz per $v = 3.2$ m/s (Yomosa)	EMf MHz per $v = 0.34$ m/s (Ichinose)	EMf KHz per $v = 0.0005$ m/s (Ichinose)
0.002	2-5	0-1	0-1	0-0	8-22	1-2	1-3
0.334	343-349	52-53	74-75	30-30	1393-1420	148-151	218-222
0.111	112-119	17-18	24-26	10-10	455-482	48-51	71-75

Plasmodium vitality. This would most likely correspond to an infrared frequency of 2-5THz. Thus interfering with this frequency from an infrared source should disrupt malaria invasion process. Although this far infrared electromagnetic frequency cannot penetrate human body more than down to 4 cm [34], such radiation can be of great help in combating *Plasmodium*, since a sizeable part of parasite remain in the skin for hours after the mosquito bite. In addition the specific RRM frequency is capable to resonantly initiate a whole cascade of protein-protein (DNA, RNA) interactions directed to the specific biological activity which could counteract *Plasmodium* infection.

Additional file

Additional file 1: Sequences submitted to RRM Analysis. (DOCX 17 kb)

Abbreviations

AMA-1: Apical Membrane Antigen 1; CS: Circumsporozoite Protein; CTRP: Circumsporozoite and TRAP Related Protein; DNA: DeoxyriboNucleic Acid; EBA175: Erythrocyte Binding Antigen 175; ICHIT: Intestinal Chitin Binding Protein; PfEMP1: Plasmodium falciparum Erythrocyte Membrane Protein 1; PfSPP: Plasmodium falciparum Signal Peptide Protease; RBC: Red Blood Cells; RNA: RiboNucleic Acid; RRM: Resonant Recognition Model; TERT: Telomerase Reverse Transcriptase; TRAP: Thrombospondin Related Anonymous Protein.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IC have introduced the RRM frequencies and electromagnetic radiation idea and have written a part and supervised the whole of the paper. JLHC has conceptualized and written part of the paper. DC has performed the calculations and analyzed the results. All authors read and approved the final manuscript.

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