Electromagnetic Properties of Biomolecules

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It is evident that macromolecular interactions involve the transfer of energy. It has been proposed in the literature that the vibrational energy transfer is unlikely to occur "because the typical time scale of vibrational relaxation (in order of few pica seconds) is much shorter than that of the resonant intermolecular vibrational energy transfer". Here we present a concept, the so- called Resonant Recognition Model (RRM), which is based on a possibility of the resonant electromagnetic (EM) energy transfer between interacting molecules. The hypothesis of the possibility of EM energy transfer instead of the vibrational one can elucidate the nature of much faster molecular interactions (higher resonant frequencies).

***Keywords**: electromagnetic field, energy transfer, resonant frequency, protein interactions*

1. INTRODUCTION

Experimental investigations of the action of electromagnetic field (EMF) at the molecular level have shown mainly resonance effects, and these are in a very wide frequency range - from low to super-high. In recent years other new mechanisms of resonance absorption of EMF in biological media have been discovered [1,2]. For instance, conformational vibrations of protein molecules consist of the formation of folds, twisting or compression of protein polypeptide chains. Conformational vibrations of protein molecule lead to the displacement of electric charges on their surface and thus, EMF of the particular frequency can interact with these vibrations [1,2]. This mechanism might underlie the resonance absorption effects due to the action of audio frequency EMF on protein solutions. Another type of the resonance absorption is in the radiofrequency (RF) spectrum, the so-called "piezoelectric resonance", was observed in some biopolymers. It was hypothesised that piezoelectric resonance is attributed to the interaction of an elastic wave produced by the EMF with defects and non-homogeneities on the surface and in the interior of the substance [1,2]. Thus, if the effects observed in biopolymers are piezoelectric in nature then biological structures must contain regions consisting of many macromolecules with the piezoelectric properties.

In general, the studies of interactions of EMF with biological media have been separated by frequency – where some research concerns with extremely low frequency (ELF) effects and others with RF exposures. Much of the research on the effects of weak EMF on organisms was concentrated on possible interactions with cell membranes [3-8]. It was proposed that cell membranes could act as non-linear resonators strongly amplified signals within a narrow range of frequencies. Interactions between EMF and bio-systems have been intensively studies for over a century and a quantitative understanding of many interaction mechanisms exists: effects arise from nerve excitations, electronically induced forces, the dielectric breakdown of cell membranes and other processes that directly involve electric fields [3-8].

Each biological process involves a number of interactions between proteins and their targets (other proteins, DNA regulatory segments or small molecules). Each of these processes involves an energy transfer between the interacting molecules. These interactions are highly selective, and this selectivity is defined within the protein primary structure. However, the physical nature of these interactions is not yet well understood. The most acceptable existing model, the so-called *key-and-lock model* that incorporates a selectivity of interactions, is based on a spatial complementarity between the interacting molecules. With knowledge of more 3-D structures of proteins and their complexes with ligands it can be observed that the spatial complementarity is not selective enough to be considered as a sole parameter able to describe the nature of protein interactions occurred in living systems.

On the other hand, there is much evidence that biological processes can be induced or modulated by induction of light of particular characteristic frequencies [3,4,9-11]. This is caused directly by the light-induced changes of energy states of macromolecules and in particular of proteins. The function of some proteins is directly connected with the absorption of visible light of defined wavelengths as in the case of rhodopsins. The strong light absorption is due to the presence of a colour prosthetic group bound to the protein, while the frequency selectivity of this absorption is defined by the amino acid sequence of the protein *per se*. In addition, there is evidence that light of a defined frequency can induce or enhance some biological processes, which are normally controlled by proteins only (i.e. cell growth and proliferation [11,12]). All these frequency selective

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effects of light on biological processes of protein activation imply that protein activation involves energies of the same order and nature as the electromagnetic irradiation of light.

Protein interactions can be considered as resonant energy transfer between the interacting molecules. This energy can be transferred through oscillations of a physical field, possibly electromagnetic in nature [13,14]. Since there is evidence that proteins have certain conducting or semi-conducting properties, a charge, moving through the protein backbone and passing different energy stages caused by different amino acid side groups, can produce sufficient conditions for a specific electromagnetic radiation or absorption. The frequency range of this field depends on a charge velocity estimated to be 7.87 x 10^5 m/s and on the distance between amino acids in a protein molecule, which is 3.8 Å. The frequency range obtained for protein interactions is 10^{13} to 10^{15} Hz. This estimated range includes IR, visible and UV light. These computational predictions were confirmed by comparison of:

a) Absorption characteristics of light absorbing proteins and their characteristic frequencies,
b) Frequency selective light effects on cell growth and characteristic frequencies of growth factors [11,13].

All these results lead to the conclusion that the specificity of protein interactions is based on the resonant electromagnetic energy transfer at the frequency specific for each interaction observed. Taking into account all these and other possible resonant effects taking place in the human body and different other leaving systems, it becomes possible for such effects of low-intensity EM radiation to occur if the energy goes through the resonant path [14-16]. Given the existence of these resonant effects, we consider here the possible interactions of the weak EM radiation and its influence on living systems.

2. METHODS

2.1 Resonant Recognition Model (RRM)

All proteins can be considered as a linear sequence of their constitutive elements, i.e. amino acids. The RRM model interprets this linear information by transforming a protein sequence into a numerical series and then into the frequency domain using digital signal processing methods, in particular the Fourier Transform (FFT) [14-16]. In the RRM the protein primary structure is represented as a numerical series by assigning to each amino acid a physical parameter value relevant to the protein's biological activity. Although a number of amino acid indices have been found to correlate in some ways with the biological activity of the whole protein [14,15], our investigations [17-27] have shown that the best correlation can be achieved with parameters which are related to the energy of delocalised electrons of each amino acid. These findings can be explained by the fact that the electrons delocalised from the particular amino acid, have the strongest impact on the electronic distribution of the whole protein. In this study, the energy of delocalised electrons (calculated as the electron-ion interaction pseudo-potential (EIIP) [28]) of each amino acid residue was used. The resulting numerical series represents the distribution of the free electrons energies along the protein molecule.

At the second stage the numerical series obtained are then analysed by digital signal analysis methods, Fourier and Wavelet Transforms, in order to extract information pertinent to the biological function. As the average distance between amino acid residues in a polypeptide chain is about 3.8 Å, it can be assumed that the points in the numerical sequence derived are equidistant. For further numerical analysis the distance between points in these numerical sequences is set at an arbitrary value $d = 1$. Then the maximum frequency in the spectrum is $F = 0.5d = 0.5$. The total number of points in the sequence influences the resolution of the spectrum only. Thus for *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 $f = n/N$ [14,15].

In order to extract common spectral characteristics of sequences having the same or similar biological function, the cross-spectral function is used. Peak frequencies in the amplitude cross-spectral function define common frequency components of the two sequences analysed. To determine the common frequency components for a group of protein sequences, we have calculated the absolute values of multiple cross-spectral function coefficients *M*, which are defined as follows:

$$|M_n| = |X_{1,n}| \cdot |X_{2,n}| \cdots |X_{M,n}| \ldots n = 1,2,\ldots N/2$$

Peak frequencies in such a multiple cross-spectral function denote common frequency components for all sequences analysed. Signal-to-noise ratio (S/N) for each peak is defined as a measure of similarity between sequences analysed. S/N is calculated as the ratio between the signal intensity at the particular peak frequency and the mean value over the whole spectrum. The extensive experience gained from previous research [14-16] suggests the value of S/N ratio of at least 20 can be considered as significant. The multiple cross-spectral function of a large group of sequences with the same biological function has been named as the "consensus spectrum". The presence of a peak frequency with the significant signal-to-noise ratio in a consensus spectrum implies that all of the analysed sequences within the group have one frequency component in common. This frequency is related to the 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.

In our previous extensive studies, the above criteria have been implemented and the following fundamental

conclusion was drawn: *each specific biological function within the protein or DNA is characterised by one frequency*. It has been shown in previous research that all protein sequences with the common biological function have common frequency component, which is a specific feature for the observed function/interaction [17-27]. This characteristic frequency is related to the protein biological function [14,15]. Furthermore, it was shown that the proteins and their targets have the same characteristic frequency in common. Thus, it can be postulated that the RRM frequencies characterise not only a general function but also a recognition and interaction between the particular protein and its target [14,15].

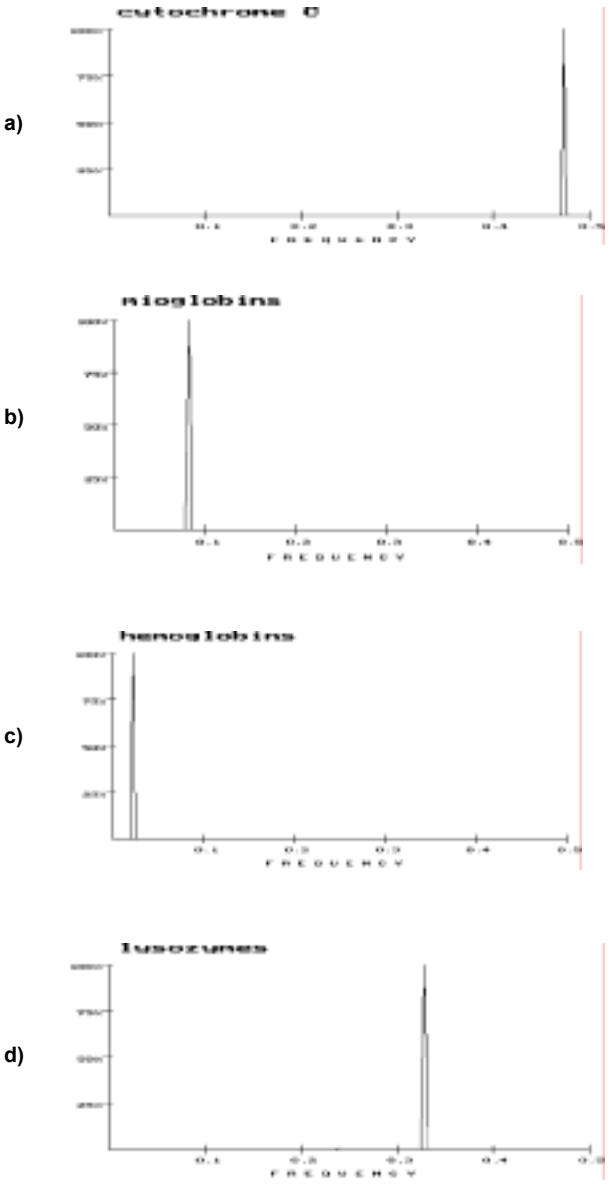

Figure 1. Example of different characteristic frequencies corresponding to different protein functions:
a) cytochrome C; b) mioglobins;
c) hemoglobins; d) lysozymes.

3. PRELIMINARY RESULTS

To grasp the meaning of the characteristic frequency it is important, at first, to understand what is meant by the biological function of proteins. As was pointed out above, each biological process is driven by proteins that selectively interact with other proteins, DNA regulatory segment or small molecules. These interactive processes that involve energy transfer between the interacting molecules are highly selective. How is this selectivity achieved? In the RRM it is postulated that the selectivity is defined within the amino acid sequence. It has been shown that proteins and their targets share the same characteristic frequency [14-16] but of opposite phase [17-20] for each pair of interacting macromolecules. Thus, we conceptualise that RRM characteristic frequencies represent proteins general functions as well as a 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. The RRM model assumes that characteristic frequencies are responsible for the resonant recognition between macromolecules at a distance. Thus, these frequencies have to represent oscillations of some physical field which can propagate through water dipoles. One possibility is that this field is electromagnetic in nature [14-16].

There is evidence that proteins and DNA have certain conducting properties [31]. If so, then charges would be moving through the backbone of the macromolecule and passing through different energy stages caused by the different side groups of various amino acids or nucleotides. This process provides sufficient conditions for the emission of electromagnetic waves. Their frequency range depends on the charge velocity, which in turn depends on the nature of charge movement (superconductive, conductive, soliton transfer, etc.) and on the energy of the field that causes charge transfer. The nature of this physical process is still unknown; however, some models of charge transfer through the backbone of macromolecules have been accepted [30-32]. To simplify the calculations we assumed the electron transfer is attributed to the difference of the free Electron Ion Interaction Potentials (EIIP) at the N and C terminals of the protein molecule. According to the theory of pseudo-potentials [28] this potential difference is:

$$W = W(\mathrm{COOH}) - W(\mathrm{NH2}) = 0.13\ Ry$$

This energy difference allows for a maximum velocity of the electrons of:

$$V_{\max} = \sqrt{2eW/m}$$

where e is the electron charge, and m is electron mass. Therefore

$$V < 7.87 \cdot 10^5\ \mathrm{m/sec}$$

As an inherent assumption is that amino acids in the protein sequence are equidistant at:

$$d = 3.8\ \mathrm{A}$$

Then, the maximum frequency that could be emitted during the electron transfer is

$$F_{\max} < V/2d$$

or

$$F_{\max} < 10^{15} \text{ Hz}$$

while the corresponding wavelength is

$$L_{\min} > 330 \text{ nm}$$

The minimum frequency that could be emitted depends on the total length of the protein

$$F_{\min} = 2F_{\max} / N$$

where N is the total number of amino acids in the protein. For example with proteins of 200 a.a. in length, the minimum frequency is

$$F_{\min} < 10^{13} \text{ Hz}$$

and the corresponding wavelength is

$$L_{\max} < 30\,000 \text{ nm}$$

The range from 30 000 nm to 300 nm is very wide, starting from the very low infrared through the visible to the ultraviolet regions [14,15].

To prove the possibility that macromolecular interactions are based on the resonant energy transfer between interacting molecules, we compared our computational predictions with the number of published experimental results. This assumption has been studied in a number of examples that include:

- Laser light growth promotion of cells using the particular frequencies of light and producing the similar effect as it would be with the presence of growth factor proteins [11-14].
- Chymotrypsin activation which was achieved by radiation of the laser light of 850-860nm only [33,34].
- Activation of highly homologous plant photoreceptors which although being very homologous absorb different wavelengths of light [9,21].
- Proteins activated by light (eg rhodopsins, flavodoxins etc.) These proteins absorb light through the prosthetic group but frequency selectivity of this absorption and consequent activation is defined by the protein sequence [35, 36].

All these results lead to the conclusion that specificity of protein interactions is based on the resonant electromagnetic energy transfer at the frequency specific for each observed interaction. The numerical frequencies obtained similarly by the RRM for various other groups of visible light-absorbing proteins are compared with their corresponding characteristic absorption frequencies in Table 1 and this linear correlation is shown in Figure 1.

A result of considerable significance is that the scaling factor between these two sets of data is almost constant about the value of $K = 200$ [14,15].

Thus, a strong linear correlation would seem to exist between the numerical characteristic frequencies defined by the RRM and the experimentally determined frequencies corresponding to the electromagnetic radiation absorption of such proteins. From this correlation it can be observed that the full range of wavelengths, which can be related to RRM characteristic frequencies, is over 400 nm.

Table 1. The RRM frequencies and characteristic absorption frequencies of different visible light-absorbing protein groups and their scaling factor, K

protein	nm	rrm	cm-1	K
cyt c	415	0.473	24096.39	196
blue	430	0.475	23255.81	204
green	540	0.355	18518.52	191
red	570	0.346	17543.86	197
hem.	14770	0.02	677.0481	295
purple	860	0.281	11627.91	241
flavodoxin	470	0.379	21276.6	178
igf	400	0.492	25000	196
fgf	441.6	0.453	22644.93	200
insulin	552	0.344	18115.94	189
growth f.	633	0.293	15797.79	185
	650	0.293	15384.62	190
pdgf	830	0.242	12048.19	200
chymotr.	851	0.236	11750.88	200
calculative	400	0.5	25000	200

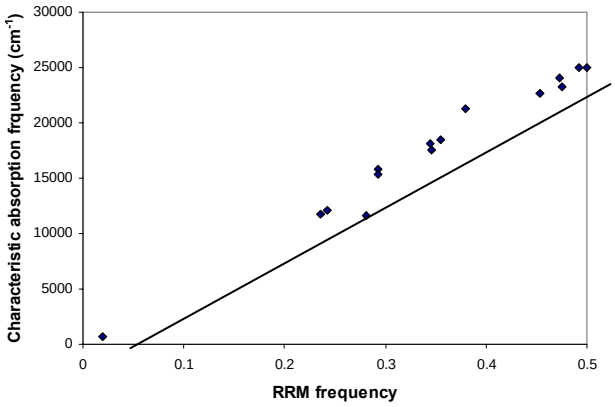

Figure 1. Linear correlation between RRM frequencies and corresponding absorption frequencies of different visible light-absorbing protein groups

This finding is in complete accord with the frequency range previously associated to the RRM spectra and calculated from the charge velocities through the protein backbone. It can be now inferred from both correlations that approximate wavelengths in real frequency space can be calculated from the RRM characteristic frequencies for each biologically related group of sequences. Furthermore, these calculations can be used to predict the wavelength of visible and near-infrared irradiation which may produce a biological effect [14,15].

As described above, the selectivity achieved within the intermolecular protein interactions is still not completely understood. There are some indications that these interactions involve a certain resonant energy transfer [1,2,37] but the physics and frequency range of

this transfer is not yet identified. In a recent work, oscillations of OH groups found on proteins and water molecules were proposed as an interaction medium for the transfer of vibrational energy from one protein to another [1, 2, 37]. However, in the case of vibrations in the condensed phase, resonant energy transfer is limited with the time scale of vibrational relaxation [1, 2, 37]. On the other hand, electronic excitations as the basis of transfer between macromolecules are more rapid. Thus, the vibrational energy transfer is an unlikely mechanism for the resonant energy transfer between the interacting macro-molecules proteins, RNA or DNA [37].

Here, we hypothesise the possibility of electromagnetic resonant energy transfer between the interacting macromolecules rather than vibrational transfer. This possibility is based on the following findings within the RRM model [14, 15]:

- The RRM frequencies are characteristic periodicities in the free electron energies along the protein or DNA molecule.
- There is a possibility of the resonant energy transfer between proteins and their DNA and protein targets, which could be relevant for protein functional specificity
- This energy could be electromagnetic in nature. The frequency range is proposed to be from infra-red up to ultra-violet (10^{13} to 10^{15} Hz)
- This energy transfer should be resonant as the frequencies in its spectrum are critical for biological function (selectivity of macromolecular interactions).
- To further investigate the proposed resonant energy transfer between macromolecules it is necessary to investigate the following aspects:
- Time scale of the proposed interaction
- Influence of water as a medium for electromagnetic radiation
- Chemical energy supplied to the activated molecule and its sufficiency to produce proposed EM radiation

Time scale of the proposed interaction could be roughly estimated by taking into account the proposed velocity of charge along the protein backbone and the linear length of the protein. For an average protein of 200 amino acids the length of the backbone would be about 800 A. The velocity of the charge is estimated to be of the order of value of 10^6 m/s which gives an estimated time for the reaction to be of the order of value of 10^{-13} s. For shorter proteins this time would be shorter (order of $2X10^{-14}$ s for proteins of 20 amino acids) and for longer proteins would be longer (up to $4X10^{-13}$ for proteins of 1000 amino acids). This time scale is of an order of value shorter than the proposed time needed for vibrational resonant energy transfer through the water dipoles [14, 15, 30-32]. The vibrational relaxation time scale is of the order of a few picoseconds (10^{-12} s) [30-32] while the time scale proposed for resonant energy transfer through electromagnetic coupling is proposed here to be of the order of value of 10^{-13} s. Although the speed of selective recognition between large linear macromolecules is not experimentally measured, there is some indication that this process, which precedes the chemical bonding, is quite quick and could be in the proposed time scale [14,15].

All these protein interactions occur in an aqueous environment and thus, it is important to investigate the water absorption characteristics for the particular frequencies relevant to a particular biological function. This is based on the assumption that photons constitute the carrier of energy from one protein to another. The depth of water required to absorb 50% of the radiation was then calculated for each of the different RRM frequencies that correspond to proteins of different biological function. The 50% point was chosen as it notionally marks the point where the probability of the emitted photon is more likely to have been absorbed by water than by another protein. Assuming that the RRM mechanism expresses its energy interchange in terms of infra-red photons, the absorption properties of water should not impede the RRM mechanism for most proteins at distances that are biologically significant. However, a group of proteins have been identified where the IR absorption properties of water may play a role, limiting the impost of biological order by this proposed mechanism to distances less than 10μm. [30-32].

In addition, the percentage of EM absorption in the water of 1μm thickness, chosen at a reasonably far distance to encompass any biomolecular interaction, is presented in Table 2. It can be observed that the majority of biological functions (except Cytochrome C) are performed at frequencies which do not have high absorbances in water. Thus, it could be concluded that water does not represent a big obstacle for EM radiation in the proposed range to be transferred from one to the other interacting molecule [30-32].

Further investigation was aimed to find out the energetics of the proposed mechanism. The energy contained in a single photon at the RRM frequency was calculated using the relationship $E = h\nu$. Then the number of photons required to produce 2*ATP was calculated. Assuming that <50% absorption of the quanta by water in the boundaries calculated above, this would leave greater than 1*ATP worth of energy for a target protein for the resonance mechanism to proceed. It is assumed, that this energy is sufficient to activate the protein to perform its function [14,15].

3.1 Applications of the RRM approach

Once the characteristic frequency for a particular protein function/interaction is identified, it is possible then to utilize the RRM to predict the amino acids in the sequence predominantly contributed to this frequency and consequently to the observed function. Also it becomes possible to predict protein active sites using the CWT and design peptide analogous having only the desired periodicities and thus, the desired bioactivity [17-27].

3.1.1 "Hot spots" in terms of the RRM and 3-D protein structures

It is known that proteins cannot express their biological function until they achieve a certain active 3D conformation. By identifying the characteristic frequency of a particular protein, it is possible to predict then which amino acids in the sequence and thus, in the 3-D structure, predominantly contribute to the frequency and consequently to the observed function [14,15,17-27]. Since the characteristic frequency correlates with the biological function, the positions of the amino acids that are most affected by the change of amplitude at the particular frequency can be defined as "hot spots" for the corresponding biological function. The strategy for this prediction includes the following steps:

- To determine the unique characteristic frequency for the specific biological function by multiple cross-spectral analysis for the group of sequences with the corresponding biological function.
- To alter the amplitude at this characteristic frequency in the particular numerical spectrum. The criterion used for identifying the critical characteristic frequency change is the minimum number of "hot spot" amino acids that are least sensitive to further changes in the amplitude of the characteristic frequency.
- To derive a numerical sequence from the modified spectrum using DFT.

It is known that a change in amplitude at one frequency in the spectrum causes changes at each point in the numerical sequence. Thus a new numerical series is obtained where each point is different from those in the original series. Detecting the amino acids corresponding to each element of this new numerical sequence can then be achieved using tabulated values of the EIIP or other appropriate amino acid parameters.

The amino acids in the new sequence that differs from the original ones reside at the points mostly contributing to the frequency. These hot spots are related to this frequency and to the corresponding biological function. The procedure described was used in a number of examples: IL-2 [13], hemoglobins, myoglobins and lysozymes [17], glucagons, TNFs [15], EGFs, FGFs [20], oncogenes [18, 27], chymotrypsins [34], and many other proteins [15, 19, 24, 26]. These examples have shown that such predicted amino acids denote residues crucial for protein functions. Consequently, these **"hot spot" amino acids are found spatially clustered in the protein's 3-D structure in and around the protein active site**.

As these specific amino acids most strongly influence the characteristic frequency, their cluster does represent a site in the protein where the signal of characteristic frequency for the specific protein property is dominant. As this cluster of amino acids has been found positioned in and around the active site (Fig.2), it is proposed that these specific amino acids play a crucial role in determining the structure of the active site and possibly, the active structure of the whole molecule [14, 15].

3.1.2 Prediction of protein active site(s) using the Continuous Wavelet Transform (CWT)

Using IFT we can identify only a number of single amino acids mostly contributed to the particular frequency. However, the protein active site is usually built up of domain(s) within the protein molecule. Applying the wavelet transform we observe a whole frequency/spatial distribution and thus, are able identify the domain(s) of high energy of a particular frequency along the sequence. The wavelet transform is an extension of Fourier transform which decomposes a signal $s(t)$ by using a set of dilated and translated function of the original mother wavelet $\psi(t)$.

The wavelet transform can be viewed as an inner product operation that measures the similarity or cross-correlation between the signal and the wavelets at different scales and translations. CWT is one of the time-frequency representations which can provide the information on how the spectral content of the signal evolves with time. The time-frequency analysis provides an ideal tool to dissect, analyse and interpret signals with transients or localised events [39].

Because CWT provides same time/space resolution for each scale and thus CWT can be chosen to localize individual events, such as the active site identification. Results of our previous studies indicated that the Morlet wavelets are the best possible wavelet functions for locating events simultaneously in both frequency and time [23, 25, 27]. Therefore in our computational studies of different protein sequences, Morlet wavelet function was chosen to identify the location(s) of active site(s) of the selected protein molecule.

The Morlet function presents a locally periodic wavetrain:

$$w(t) = c\exp(\frac{-t^2}{2} + j\omega_0 t)$$

where $\omega_0 = 5$ and c is the constant used for normalization.

Strictly speaking, the CWT provides a time-scale representation rather than a time-frequency representation. However, the scale factor of CWT is closely related to the frequency and this makes the mapping from time-scale representation to time-frequency representation possible. The active sites along the protein sequence are determined through studying the set of local extrema of the moduli in the wavelet transform domain. Those energy concentrated local extrema are the locations of sharp variations points of the EIIP and are proposed as the most critical locations for protein's biological functions.

The wavelet approach incorporated into the RRM aiming at improvement of the protein active site prediction scheme has been tested on a number of different protein groups [23, 25, 27, 40-43].

3.1.3 Bioactive peptide design

Knowing the RRM characteristic frequencies and corresponding phases for particular biological functions, it is possible to design amino acid sequences having those spectral characteristics only. It is expected the designed peptide will exhibit the desired biological activity. The strategy for the design of such defined peptides is as follows:

- Within the multiple cross-spectral analysis of the group of protein sequences sharing the corresponding biological function, determine the unique RRM frequency that characterises this specific biological function/interaction.
- Define the characteristic phases at the characteristic frequencies for the particular protein that is chosen as the parent for agonist/antagonist peptide design.
- From the known characteristic frequencies and phases derive a numerical sequence. This can be done by summing sinusoids of the particular frequencies, amplitudes and phases. The length of the numerical sequence is defined by the appropriate frequency resolution, and the required peptide's length.
- To determine the amino acids that corresponds to each element of the new numerical sequence. It can be achieved by the tabulated EIIP or other appropriate amino acid parameters.

In our previous extensive research the above mentioned procedure has been applied on different protein examples [20, 22, 27] aiming at the design of peptide analogous having the functional activity as their parent proteins.

3.2 Experimental investigation of the mechanisms of electromagnetic field interaction with proteins

It was postulated that the protein function is directly related to the absorption of light of defined wavelength. Within the RRM it was found that a strong linear correlation exists between the predicted and experimentally determined frequencies corresponding to the absorption of electromagnetic radiation of such proteins [15, 34]. It is inferred that approximate wavelengths in real frequency space can be calculated from the RRM characteristic frequencies for each biologically related group of sequences. These calculations can be used to predict the wavelength of the light irradiation, which might affect the biological activity of proteins exposed [15, 34].

Our experimental study [38] consists of the series of experiments confirming the possibility that protein activity can be influenced by external electromagnetic radiation as predicted by the RRM. Here we have monitored the chemical process involving L-Lactate Dehydrogenase (rabbit muscle) by analysis of the change in its kinetics under the influence of external EMF radiation.

L-Lactate dehydrogenase (LDH) catalyses the following reaction:

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+ + \text{H}^+$$

The suitability of LDH enzyme for this reaction is attributed to the absorption characteristics of NADH (Nicotinamide Adenine Dinucleotide, Reduced form). NADH is able to absorb the light at 340 nm in contradict to the NAD (Nicotinamide Adenine Dinucleotide Nicotinamide Adenine Dinucleotide, Oxidised form), which is inactive at this frequency. Due to the different optical characteristics of NADH and NAD we are able to optically asses if the reaction Pyruvate $\rightarrow$ Lactate in the presence of LDH as an accelerator has occurred and then determine amount of the reactants. The reaction rate depends on the concentration of enzyme and substrate [38].

As mentioned above a linear correlation between the absorption spectra of proteins and their RRM spectra with a regression coefficient of $K = 201$ and predetermined frequency range was established [14,15]. Thus, based on the characteristic frequency determined for the whole dehydrogenase functional group, we can calculate the wavelength of irradiation, λ, which assumingly would activate protein sequences and modify their bioactivity:

$$\lambda = 201 / f_{\text{RRM}}$$

The dehydrogenases' characteristic frequency is identified at $f = 0.1680$; the wavelength of the electromagnetic exposure required for dehydrogenase enzymes activation is 1196 nm.

We were not able to irradiate the selected enzyme sample by EMF of this required wavelength due to the limitations of Monochromator Spex 270 (Instruments CA, Inc) with a range of 400-890 nm. To solve this problem we decided to look at the single spectrum of the studied LDH enzyme (1.1.1.27 rabbit muscle) that is shown in Fig. 3. The RRM characteristic frequency of this enzyme is identified at $f_{\text{RRM}} = 0.3066$ that corresponds to $\lambda = 656$ nm.

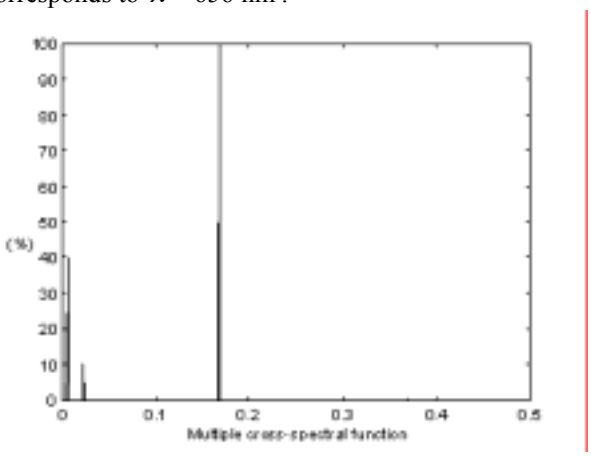

Figure 2. Multiple cross-spectral function of Dehydrogenase proteins (72 sequences). The prominent peak(s) denote common frequency components. The abscissa represents the RRM frequency and ordinate is the normalized signal intensity

Therefore, to test the concept of the possible affect of EMF on enzyme activity here we are used the external radiation in a range of 550-850. The results of this preliminary study clearly demonstrated the change of absorbance of NADH sample under the influence of irradiated LDH [38].

The results obtained have shown that NADH concentration corresponds to the maximum absorbance of 1.2 at 340 nm. Fig. 3 shows how NADH sample absorbance is affected by the applied radiation of the defined wavelength.

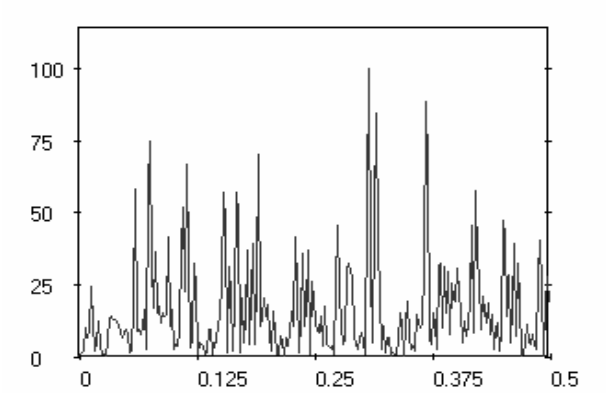

Figure 3. Single spectrum of LDH (1.1.1.27 rabbit muscle)

After being exposed to EMF in the range of 550-850nm, the LDH bioactivity has increased resulting in accelerating the reaction $\text{Pyruvate} + \text{NADH} \rightarrow \text{Lactate} + \text{NAD}^+ + \text{H}^+$.

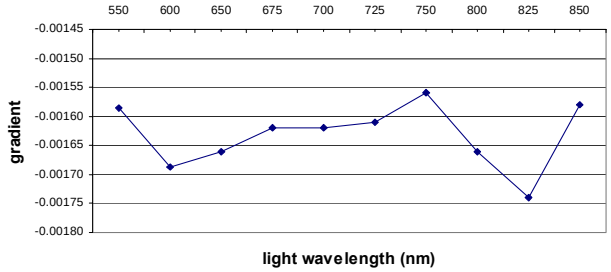

Figure 4. Gradient of change in absorbance of the solution

From Fig.3 we can clearly observe that the frequency $f = 0.3066$ ($\lambda = 656$ nm) determined by the RRM as the possible frequency corresponding to the activation of the enzyme sample, does influence on its biological function. Hence, the results reveal that this specific biological process can be modulated by irradiation with defined frequencies strongly supporting the main concept of the RRM methodology. Moreover, if we observe the increase of LDH activity at 650-675nm, it is expected that much stronger effect in protein activation will be gained if we could perform the experiment with the predicted by the RRM characteristic frequency $f = 0.1680$ ($\lambda = 1156$ nm) that correspond to the common dehydrogenase activity. Such study would be the next step of our research of protein interactions with EMF.

To summarise the results of our experimental study [38] it should be pointed out that the concept of the possible affect of EMF on enzyme activity has been investigated here with the applied source of external radiation in a range of 550-850. The results of the study clearly demonstrated:

1. The change of absorbance of NADH sample under the influence of irradiated LDH. After being exposed to EMF in the range of 550-850 nm, the LDH bioactivity has increased resulting in accelerating the reaction $\text{Pyruvate} + \text{NADH} \rightarrow \text{Lactate} + \text{NAD}^+ + \text{H}^+$.
2. The frequency $f = 0.3066$ ($\lambda = 656$ nm) determined by the RRM as the possible frequency corresponding to the activation of the enzyme sample, does influence on its biological function.
3. Hence, the results reveal that this specific biological process can be modulated by irradiation with defined frequencies strongly supporting the main concept of the RRM methodology.
4. Moreover, if we observe the increase of LDH activity at 650-675 nm, it is expected that much stronger effect in protein activation will be gained if we could perform the experiment with the predicted by the RRM characteristic frequency $f = 0.1680$ ($\lambda = 1156$ nm) that correspond to the common dehydrogenase activity. Such study would be the next step of our research of protein interactions with EMF.

5. CONCLUSION

In our extensive research so far we have shown that:

- Certain periodicities (frequencies) within the distribution of the free electron energy along the protein and DNA regulatory sequences are related to the biological function of these macromolecules;
- These characteristic frequencies are the same for interacting pairs of molecules indicating the possibility of resonant recognition between them;
- If charge transfer along the protein (DNA) backbone is assumed, then these frequencies could be frequencies of electromagnetic radiation or absorption within the macromolecule.
- The frequency range of this EM radiation was estimated to be within IR, visible and partially UV light radiation.
- When this assumption was tested in a number of light activated proteins or processes, a linear correlation was obtained.
- It has been shown that this electromagnetic radiation can propagate through the water with small losses for most characteristic frequencies.
- Energetics of these processes was calculated and it has been shown that in most cases one photon carries enough energy to excite the next molecule. However, energy from ATP would not be enough to activate the radiation process.

- The speed of the process was estimated to be approximately of 0.1 pica sec, order of value faster than the vibrational relaxations in the molecule.
- The possibility to change/increase of LDH enzyme activity by light irradiation of the computationally defined RRM frequency was experimentally investigated.

Having this entire in mind, we can conclude that there is a possibility of electromagnetic resonant energy transfer between proteins and their protein or DNA targets which could be relevant to their biological function. However, to prove these concepts thoroughly more in-depth experimental and theoretical studied are required to be undertaken.

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ЕЛЕКТРОМАГНЕТНЕ КАРАКТЕРИСТИКЕ ПИОПОЛИМЕРА

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Евидентно је да макромолекуларне интеракције укључују пренос енергије. У литератури је предложено да је вероватноћа вибрационог трансфера занемарљива зато што је временска димензија вибрационе релаксације (реда неколико пикосекунди) много краћа него што је време резонантног "вибрационг трансфера енергије", [36,37]. У раду је презентиран концепт такозваног резонантног препознавања *Resonant Recognition Model (RRM)* који је базиран на вероватноћи резонантног електромагнетског трансфера енергије између интерагујућих молекула. Такође електромагнетним преносом енергије се могу објаснити врло брзе молекуларне интеракције које не објашњава вибрациони пренос енергије.