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Biological effects of electromagnetic radiation

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1. Introduction

There are a considerable number of peer-reviewed publications which show that electromagnetic EMR can result in physiologically beneficial *in vivo, in vitro* and *in situ* biological effects. These bioeffects are already used for therapeutic applications of non-thermal and non-ionizing EMR such as bone repair, nerve stimulation, wound healing, osteoarthritis treatment, electro-acupuncture, tissue regeneration, immune system stimulation, and neuroendocrine modulations.

Pulsed electromagnetic fields, pulsed radio frequency fields, and low frequency sinusoidal waveforms have been shown to promote healing when used as adjunctive therapy for a variety of soft-tissue injuries. It was also shown that specific changes in the field configuration and exposure pattern of low-level EMR can produce highly specific biological responses (Breckenkamp et al., 2003; Kappe and Stadler, 2005; Frochlich, 1984; Jauchem, 2008; Kundi, 2008; Sheppard et al., 2008; Vojisavljevic et al., 2007).

It is widely accepted that electromagnetic pollution is a major environmental factor which is currently not well investigated. The ever-increasing number of mobile phones, base stations, digital mobile communication systems, and other portable devices operating at radio frequencies (RF), motivates the scientific community to better understand the mechanism of interaction between radio/microwave radiation and biological systems. Environmental and occupational health risks are increasingly a focus of public concern. Since all living organisms are exposed to electromagnetic radiation, it is important to establish standards of acceptable exposure levels for a comprehensive range of RF radiation. At present there is no substantiated scientific evidence to indicate adverse health and biological effects produced by RF exposures at levels below national and international safety standards.

Studies on human subjects have shown consistently that there is no evidence that prolonged exposure to weak electric fields results in adverse health effects (Eberhard et al., 2008; ARPANSA, 2008; ICNIRP, 1998). However, the question of whether chronic exposure to weak magnetic fields is equally harmless remains open (Eberhard et al., 2008). Laboratory studies on animals and cell cultures have shown that weak magnetic fields may have effects

on several biological processes. For example, they may alter hormone and enzyme levels and the rate of movement of some chemicals through living tissue (ARPANSA, 2008). Although these changes do not appear to constitute a health hazard, they need further investigation particularly of the long term affects, and the possible mechanisms of any effects (ICNIRP, 1998).

Some effects of RF irradiation on biological processes can be reversible and thus, do not impact the health of humans. However, the significance of investigating non-thermal RF biological effects at the molecular level should not be underestimated. Understanding the mechanism of interaction between RF radiation and molecules, determining exposure level and duration (dosimetry protocol) that can result in irreversible changes in biological processes will lead to better understanding of health effects in people due to exposure to RF irradiation.

The effects of RF irradiation on global gene and protein expression in different biological systems have been investigated (Hardell & Sage, 2008) and most studies focused on the mobile phone frequencies (800 MHz –2000 MHz) at a relatively low exposure density (average SAR near 2.0 W/Kg). Based on current literature, it can be summarised that RF exposures can change gene and/or protein expression in certain types of cells, even at intensities lower than the standard recommended exposure levels (Hardell & Sage, 2008). However, the biological consequences of most of the changed genes/proteins are still unclear, and need to be further explored.

Currently the body of knowledge in this area is not sufficient to assess the impact on health due to RF irradiation, considering existing gene and protein expression data. With only a limited number of studies reporting often conflicting results (Hardell & Sage, 2008) the electromagnetic radiation (EMR) research community should pay equal attention to the negative reports as to the positive ones. Not only the positive reports need to be replicated, the negative ones need to be critically assessed and replicated as well (Hardell & Sage, 2008).

One powerful approach to the problem of the unknown effects on proteins of non-thermal RF exposures, is studying the effect of irradiation on protein activity (molecular level of interaction). Proteins are the macromolecules found in a living cell and play a crucial role in almost every biological process. The effects of electromagnetic fields (EMF) at different frequencies of exposure on proteins were analysed within our studies with the aim of understanding the possible mechanisms of interaction between external electromagnetic radiation and biological media.

We focused on enzymes, which are a specific group of proteins crucial in accelerating metabolic reactions in living organisms. In other studies isolated aqueous enzyme solutions were also taken as models to determine if external radiation could influence the selected biological processes (Vojisavljevic et al., 2007; Karu et al., 1999; Vorst et al., 2006; Dutta et al., 1992; Pavicic &Trosic, 1997; Peinnequin et al., 2000). By using isolated enzymes, many of the complexities associated with whole-body irradiation can be eliminated. By undertaking this research we intent to address the issues of biological effects and possible health hazards associated with exposure to RF radiation which are of growing concern to military,

industrial and government organizations. We aimed to approach the question of biological effects of low intensity RF exposures in a methodical and quantifiable way.

2. Background

The actual mechanism by which EMFs produce biological effects is under intense study. The ideas of resonant absorption and resonant interactions have been proposed as an explanation for the marked sensitivity of living systems to EMF (Frochlich, 1986; Cosic, 1994; 1997). Evidence suggests that the cell membrane may be one of the primary locations where applied EM fields act. EM forces at the membrane's outer surface could modify ligand-receptor interactions, which in turn would alter the state of large membrane molecules that play a role in controlling the cell's internal processes (ICNIRP, 1998). The electropotential of healthy cells causes a steady flow of ions across the cell membrane which could be disturbed in an unhealthy or damaged cell. Studies have shown that the application of EMR to damaged cells can accelerate the re-establishment of normal potentials, increasing the rate of healing, reducing swelling, and helping to reduce bruising. Faster healing times will provide significant cost savings and improved patient care. The following studies have demonstrated accelerated EMR effects at the cellular and molecular level:

1) Results of several double-blind studies showed that pulsed EMF (PEMF) stimulation promotes cell activation and proliferation by an effect on cell membranes, particularly endothelial cells (Stiller et al., 2006).
3) Extremely Low Frequency (ELF) and RF fields have been applied to accelerate wound healing. Since skin wounds have unique electrical potentials and currents, stimulation of these electrical factors by a variety of EMFs can aid in the healing process by causing dedifferentiation (i.e., conversion to a more primitive form) of the nearby cells followed by accelerated cell proliferation (Teper et al., 2004).
4) PEMF increases the rate of formation of keratinocytes cells in partially healed skin wounds (Vianale et al., 2008).

Light activated changes in protein energy states have been shown to induce or modulate biological processes. For example light-activated excitation of the rhodopsin/ bacteriorhodopsin molecules involved in the hyperpolarisation process of the cell membrane can either generate nerve impulses, ATP synthesis, or light regulated embryogenesis (i.e. seed germination in plants) (Ahma et al., 1993; Karu, 1987; 1999). It has also been suggested that cytochrome C oxidase and certain dehydrogenases may play a key role in the photoreception process, particularly in the near infra-red (NIR) frequency range (Karu 1999). A number of different investigations (Karu, 1987; 1999; Fedoseyeva, 1988) have explored the effects of visible light on cell proliferation and metabolism. Some recent studies into effects of low-intensity non-thermal light irradiation on eukaryotic and prokaryotic cells, both pulsed and continuous, have reported positive results such as accelerated proliferation rate in yeast and mammalian cells after irradiating with a He-Ne laser (Karu, 1987; 1999; Karu et al., 2004) and increased *E. coli* proliferation during argon laser light exposure. An increased proliferation rate has also been observed in various bacterial cultures irradiated by laser light with radiant exposures of 1-50 J/cm² at wavelengths of 630 nm and 810 nm (Nussbaum, 2002). The stimulating effect of various light-emitting diodes

(LEDs) and monochromatic light on eukaryotic cells have been documented with pulsed and continuous light exposures. Several studies reported a change in acetylcholinesterase activity of human erythrocytes after low-intensity light radiation at 810 nm (Kujawa et al., 2003)

In our studies we investigated the influence of non-thermal non-ionizing electromagnetic radiation on L-Lactate dehydrogenase enzyme kinetics using the concepts of protein activation outlined in the Resonant Recognition Model (RRM). The RRM approach postulates that selectivity of protein interactions is based on specific resonant electromagnetic interactions (Cosic, 1994; 1997).

Previously (Cosic, 1997; Cosic & Birch, 1994; Cosic, 1989) a relationship between the RRM spectra of some protein groups and their interaction with visible light was established. The RRM theory states that an external EMF at a particular activation frequency would produce resonant effects on protein biological activity. It has been shown that all protein sequences with a common biological function have a common frequency component in the free energy distribution of electrons along the protein backbone. This characteristic frequency was shown to be related to protein biological function (Cosic, 1994; 1997). Furthermore, it was also shown that proteins and their targets share a characteristic frequency. It was postulated that RRM frequencies characterise not only a general function but also a recognition/interaction between the particular proteins and their target at a distance (Cosic & Hearn 1991; 1992). Thus, according to the RRM protein interactions can be viewed as a resonant energy transfer between the interacting molecules (Cosic, 1994; 1997).

Comprehensive computational analysis of different light absorbing proteins using the RRM approach showed that a strong linear correlation exists between the predicted and experimentally determined frequencies corresponding to the absorption of electromagnetic radiation of such proteins (Cosic, 1994). 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 exposed proteins (Cosic, 1997). These computational predictions were confirmed by comparison of:

a) Absorption characteristics of light absorbing proteins and their characteristic RRM frequencies (Cosic, 1997; Cosic & Birch, 1994; Cosic et al., 1989).
b) Frequency selective light effects on cell growth and characteristic RRM frequencies of growth factors (Cosic et al., 1989).
c) Activation of enzymes by laser radiation (Biscar, 1976; Vojisavljevic et al., 2007).

All these results indicate that the specificity of protein interaction is based on a resonant electromagnetic energy transfer at the frequency specific for each interaction observed. A linear correlation between the absorption spectra of proteins and their RRM spectra with a regression coefficient of K=201 has been established. Using the RRM postulates, a computationally identified characteristic frequency for a protein functional group can be used to calculate the wavelength of applied irradiation, λ, which assumingly would activate this protein sequence and modify its bioactivity (Cosic et al., 1989):

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

In our study we utilised this relationship to calculate the frequencies/wavelengths that might modulate the bioactivity of the selected LDH enzyme. Its activation using the external EMR was investigated experimentally. The influence of visible light, infra-red and low level microwaves and their modulating effects on enzyme kinetics were evaluated.

Study	Exposure	Frequency	Effects
(Dutta et al., 1991)	0.3-8.0V/m	147 MHz (modulated with 16 Hz)	Exposures to modulated RF radiation with different powers produce different effects on Accetilholesterase activity
(Pavicic & Trosic, 2008)	0.3-8.0V/m	864 MHz; 935 MHz	RF irradiation significantly affected cell proliferation kinetics
(French et al. 1997)	200-400 V/m	835 MHz	The EMF at 835 MHz and power densities similar to the exposures emitted by mobile phones can alter cell morphology and inhibit the proliferation of Astrocytoma cells.
Peinnequin et al. 2000	137.3 V/m	2.45 GHz	RF interacts either with Fas pathway between receptor and caspase-3 activation or with membrane proteins
(Tian-Yong Zhao, 2007)	Mobile phone	900 MHz – 1800 MHz	Exposure to cell phone RF radiation emissions can up-regulate the specific intermediaries of apoptotic pathways of neurons.
(Fesenko et al., 1995)	86 V/m	42.2 MHz	The data suggest that the effects of the field on the ion channels are mediated, at least partially, by changes in the solution properties.
(Porcellia et al. 1997)	High Power	10.2 GHz	Conformational changes of S-adenosylhomocysteine hydrolase.
(Byus et al. 1984)	10mW/cm²	450 MHz (modulated 60 Hz)	Large reduction in histone kinase activity in lymphocytes.

Table 1. Biological effects of low power radiofrequency irradiation

Doubt remains about the existence of non-thermal biological effects. Non-thermal biological effects are measurable changes in biological systems that may or may not be associated with

adverse health effects. Thirty years ago, Frohlich suggested that extremely weak microwave signals may act as a trigger for certain bioeffects (Frohlich, 1986). For example, non-thermal effects such as: calcium ion efflux; changes in blood-brain barrier permeability; changes in melatonin level; and alterations in EEG signals have been observed at low-intensity modulated RF fields Little is known about the molecular mechanisms involved in putative non-thermal effects. One hypothesis is that RF radiation can induce dipole oscillations in a protein active site and thus can alter its function (Kape &Stadler, 2005). This hypothesis accords well with observed increased permeability of aqueous protein solutions measured using dielectric spectroscopy at the frequency range of 10 MHz – 1000 MHz (Dutta et al., 1992).

The use of radiofrequency (RF) sources increased dramatically in the last century particularly with the introduction of radio, television, mobile communication and other specific applications in medicine and industry. Although the level of radiation from these RF sources is well below exposure levels where thermal effects are induced in biological systems, there is still intense debate about the impact of RF radiation on human health. Two types of effects can be ascribed to RF fields, i.e. thermal and non-thermal. The heating effect of radio waves is already well-known and documented. The thermal effects are related to the heat generated by the absorption of RF energy by the water medium or by organic complex systems (Vorst et al., 2006). It has been known for many years that exposure to very high levels of RF radiation can be harmful due to the ability of RF energy to rapidly heat biological tissue (Vorst et al., 2006).

The first studies into possible non-thermal biological effects were carried out using various commercial/industrial RF apparatuses working at their particular frequencies and powers of radiation (Duta et al., 1992, Vorst et al., 2006). Researchers have had difficulty reproducing the results of these studies and have not been able to determine the reasons for this inconsistency. Note that only selected frequency exposures emitted by radiofrequency equipment (microwave ovens, radars or mobile phones) have been tested on selected cells and proteins (Table 1) whereas this study has covered a comprehensive range.

3. Experimental study

3.1 Effects of visible light and infrared radiation on enzyme activity

In this study we demonstrated that visible light and infrared (IR) radiation at specific frequencies in the range of 550 nm – 850 nm and 1140 nm - 1200 nm respectively can modulate enzyme activity (Vojisavljevic et al., 2007; Pirogova et al., 2008).

Dehydrogenase enzymes catalyse a variety of oxidation-reduction reactions within the cells. Aiming to avoid any ambiguities and inconsistencies in interpretation of the experimental findings, the enzyme example, LDH (EC1.1.1.27, rabbit muscle), has been selected on the basis its commercial availability, simplicity of the enzymes assay, and the possibility of measuring its bioactivity using the standard well accepted procedure, i.e. Continuous Spectrophotometric Rate Determination. LDH catalyses the inter-conversion of the l-lactate into pyruvate with the nicotin amide adenine dinucleotide (NAD+) acting as a coenzyme. The suitability of the LDH enzyme for this reaction is attributed to the absorption

characteristics of the NADH (Nicotinamide Adenine Dinucleotide, Reduced form). NADH is able to absorb 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 the NADH and NAD we are able to optically asses if the reaction Pyruvate → Lactate in the presence of the LDH as an accelerator has occurred and then determine the amount of the reactants. The reaction rate depends on the concentration of the enzyme and substrate.

As a source of near IR and visible light we used a SPEX 270M monochromator with a wavelength range of 400-890 nm and a resolution of 0.1 nm. For measurement of absorbance of the analysed enzyme solutions we will use an Ocean Optics USB2000 spectrometer coupled to a CCD array which can detect in the 190-870 nm range. Software control automatically monitors and saves the absorption coefficient at the required wavelength every 30 sec.

Enzyme activity measurement
The assay contained the following components: 3.00 ml reaction mix includes 10 mM sodium phosphate, (SIGMA, St Louis, MO USA) 0.12 mM –NADH, disodium salt (ROCHE, Roche Diagnostics GmbH Nonnenwald 2 DE-82377 Penzberg GERMANY) reduced form, 2.3 mM pyruvate,(BioWhittaker™ Cambrex Bio Science Walkersville, MD USA) 0.033% (w/v) of bovine serum albumin (SIGMA, St Louis, MO USA) and 0.05 units of L-lactic dehydrogenase (ROCHE, Roche Diagnostics GmbH Indianapolis In, USA).

The LDH kinetics was measured by the continuous monitoring of the NADH absorption at 340 nm. The activity of the enzyme examples is determined by the rate of substrate utilization during the enzyme-catalysed reaction. The temperature was controlled during the enzyme solution irradiation as well as during the activity measurement procedures. Enzyme concentration was determined by the extinction coefficient. For each irradiated sample we measured the absorption spectra for 10 min by recording the solution's absorption values every 30 sec as follows:

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right)$$

where S_λ is the sample intensity at wavelength λ defined as an intensity of incident light that passes the cuvette with the sample, D_λ is the dark intensity at wavelength λ, or intensity measured on the surface of the CCD detector (spectrometer) when the light source is switched off. R is the reference intensity at the wavelength λ. The activity rate was determined by calculating the gradient of $A_\lambda(t)$.

It was observed that during the first 10 min the increase of the absorbance is a linear process and can be calculated as a value of the gradient of the line representing the relation between the absorbance and time. For each wavelength of light radiation we undertook 3-5 control experiments to measure the activity of non-radiated protein solutions. With the aim at eliminating the effect of all possible artefacts we repeated our measurements with the irradiated enzyme example at the particular wavelengths of light that correspond to the

resonant effects. Also we have randomised an order of measurements (596nm, 844nm, 724nm etc.) to eliminate a possible modification of the enzyme or substrate during the experimental procedure. The results obtained were analysed and the enzymatic activity of non-radiated solutions was compared to the activity of the irradiated samples (calculation of the P value). To evaluate a significance of differences between the mean values of the activity of irradiated and non-irradiated samples, we used an independent two-sided T-test.

3.2 Effects of low level microwaves on enzyme activity

In the next study we investigated the effects of low level RF irradiation on LDH enzymatic activity. The activity of LDH was calculated from absorption curves using the "gradient methods" (Vojisavljevic et al., 2007). The enzyme solutions were exposed to ultra high frequency (UHF) electromagnetic radiation using a TC-5062A UHF TEM Cell, TESCOM Ltd (TC-5060 Operating Manual). The frequencies of applied radiation were in the range 400 MHz - 975 MHz and enzyme activity was monitored (by measuring optical density of the LDH solution) at steps of 25 MHz.

For each frequency of irradiation the effect was measured for the following electrical fields: 0.02135 V/m, 0.06754 V/m 0.21358 V/m 0.675 V/m and 2.136 V/m (0.0000012 W/m^2, 0.000012 W/m^2, 0.00012 W/m^2, 0.0012 W/m^2, and 0.012 W/m^2 respectively). Measurements were repeated 3-8 times depending on their standard deviations, irradiating frequency and electrical field values.

## 4. Results and Discussion

4.1. Visible light irradiation

In order to computationally predict the activation frequency/wavelength of light irradiation that could produce significant effects on LDH function, a database of 176-LDH protein sequences was established and analysed using the RRM approach. All protein sequences have been taken from SWISSPROT database. Accordingly to their different structural origins and functions the selected LDH sequences were divided onto 4 sub-groups: LDH_A, LDH_B, LDH_C and bacterial LDH. The cross spectral functions were calculated for each of these groups and are shown in Figure 1a,b,c,d.

The characteristic frequencies of the LDH enzymes were identified at the following prominent frequencies: **0.1688, 0.332 ± 0.08 and 0.242 ± 0.08**, which correspond to the light radiation with a wavelength of **1190 nm, 605 nm ± 10 nm and 830 nm ± 12 nm** respectively. The RRM frequencies **0.332 ± 0.08 and 0.242 ± 0.08** are particularly prominent in the LDHB cross-spectral function. While for the LDH_A, LDH_C and bacterial LDH amplitude ratio of the peaks at the frequencies **0.332 ± 0.08 and 0.242 ± 0.08** are significantly smaller (Fig.1).

Based on the characteristic frequencies determined for the whole dehydrogenase functional group, we have calculated the wavelength of irradiation, $\lambda=201/f_{RRM}$ which assumingly would activate dehydrogenase sequences and modify their bioactivity. Thus, the wavelengths of the electromagnetic exposure required for dehydrogenase enzymes activation would be at **1191± 15 nm and 846±15 nm.**
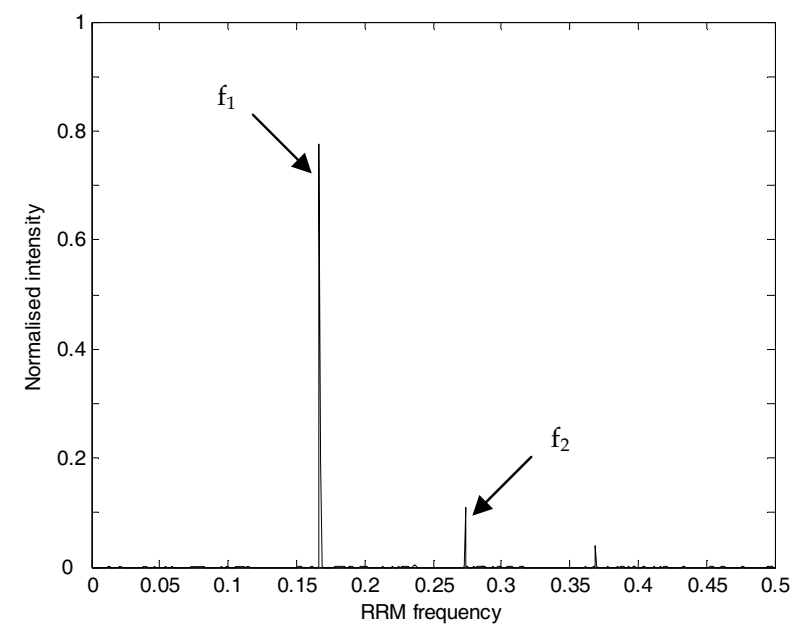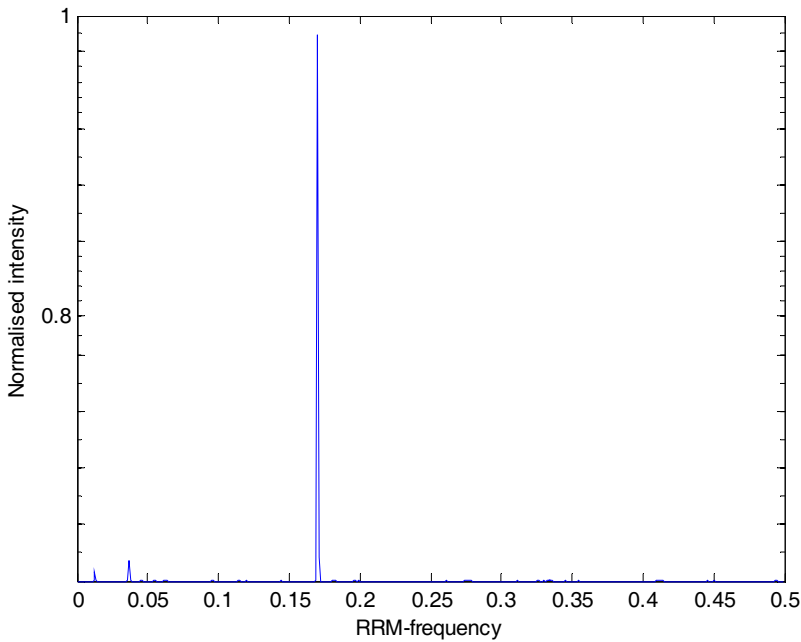
(a)
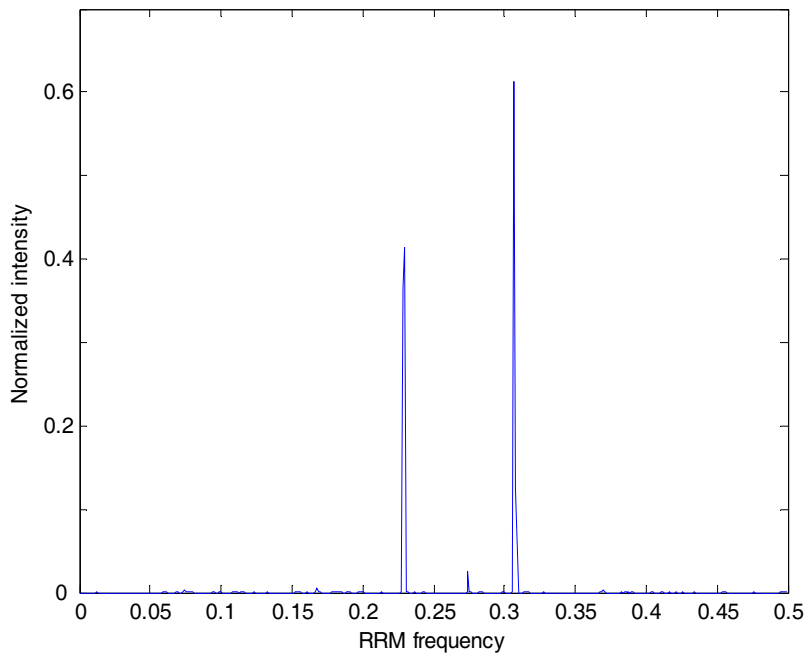
(b)
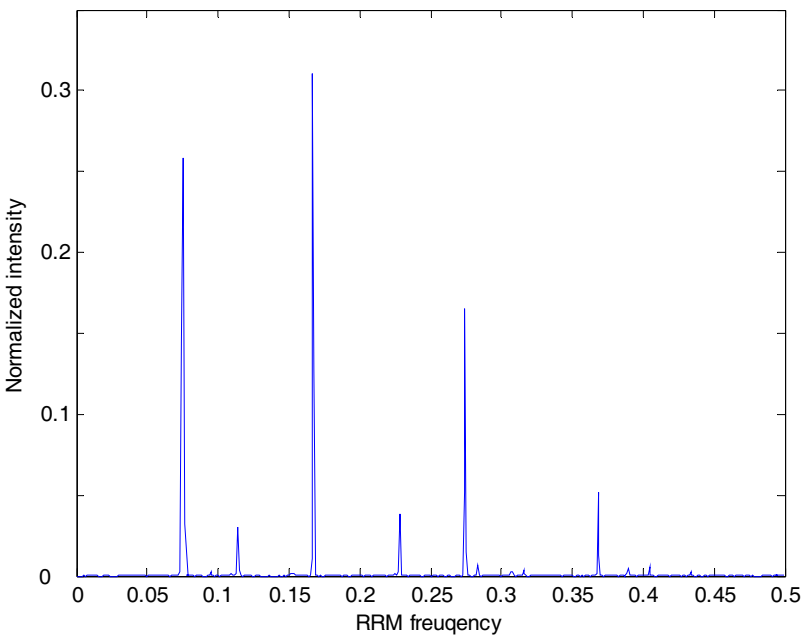
(c)

(d)

Fig. 1. Multiple cross spectral function of (a) LDH_A sequences, (b) LDH_B sequences, (c) LDH_C sequences and (d) LDH_bacterial. The x axis represents the relative RRM frequency and corresponding wavelength of the electromagnetic radiation. The y axis represents the normalized intensity of the multiple cross spectral function in the RRM spectra.

Therefore, we investigated the effects of visible light in the range of 550 nm - 850 nm and infra-red ranging from 1140 nm to 1200 nm on the LDH kinetics (Vojisavljevic et al., 2007; Pirogova et al., 2008).

Data were collected and presented in Figure 2. The effects of light exposures on the L-LDH activity are measured as the rate of change of the NADH concentration per second. There is the evident increase in the L-LDH activity after irradiation by visible light at the particular wavelengths: 829 nm and 595 nm (Figure 2). The enzyme solutions were irradiated for 15 min. The enzyme activity measured immediately after irradiation at 829 nm is 0.025 ± 0.001 and at 595 nm is 0.025 ± 0.001 respectively.

However, there is no significant difference in activity observed between LDH example, which was radiated by light of other wavelengths, and activity of the control non-radiated solutions. In comparison to the non-radiated LDH solutions that have average rate of 0.022 with a standard deviation of ± 0.0015, the results obtained demonstrate the increase of LDH activity in order of 11.9% ($P < 0.001$) at 596 nm and 12.67% ($P < 0.001$) at 829 nm respectively.

To evaluate how significant is the difference between the mean values of the activity of irradiated and non-irradiated samples, we have used an independent two-sided T-test. This value is much higher than the value of the variability caused by the variations in concentrations of the LDH, NADH and pyruvate that were determined within the control experiment with the non-irradiated solutions. It is important to note, the increase of biological activity achieved upon radiation by light of the particular wavelengths at 596 nm and 829 nm has been already reported in literature.

Study of the effect of burn healing in non-diabetic rats produced by LED light at 596 nm was published (Al-Watban & Andres, 2003). Also light radiation at 829 nm is close to the 810 nm that was proved to be effective for bacteria's growth rate. In particular, the low-level laser at 830 nm has produced the effects in control of the painful stomatitis in patients with hand-foot (Toida et al., 2003).

Summarising the results of our study we have to point out that the computationally predicted activation frequencies of the LDH enzymes using the RRM approach closely correspond to our experimental data and findings of other researchers showing the maximum change in the LDH activity after irradiation at 596 nm and 829 nm.

Hence, the results reveal that this specific biological process can be modulated by irradiation with the defined frequencies strongly supporting the main concept of the RRM methodology. Moreover, if we observe the increase of the LDH activity at 550-900 nm wavelength range, it is expected that much stronger effect in protein activation would be obtained if we could perform the experiment with the predicted by the RRM characteristic frequency f=0.1680 (λ=1156nm) that correspond to the common dehydrogenase activity. Such study would be the next step of our research of protein interactions with the EMF.

4.2 Infra-red radiation

Measurement of the NADH absorbance

1. We have diluted the stock coenzyme solution with the 0.003 M potassium phosphate assay buffer.

2. Using the properly diluted coenzyme solution, we have measured the NDAH absorbance after irradiation at 1140 - 1200 nm with the interval of 2-5 nm.

3. The spectrophotometer was set to 100% transmittance (zero absorbance) at each wavelength using 0.003 M K_2HPO_4 assay buffer blank. The results reveled that NADH concentration corresponds to the maximum absorbance of 1.6 at 340 nm. Fig. 3 shows how NADH sample absorbance is affected by the applied radiation of the defined wavelength.
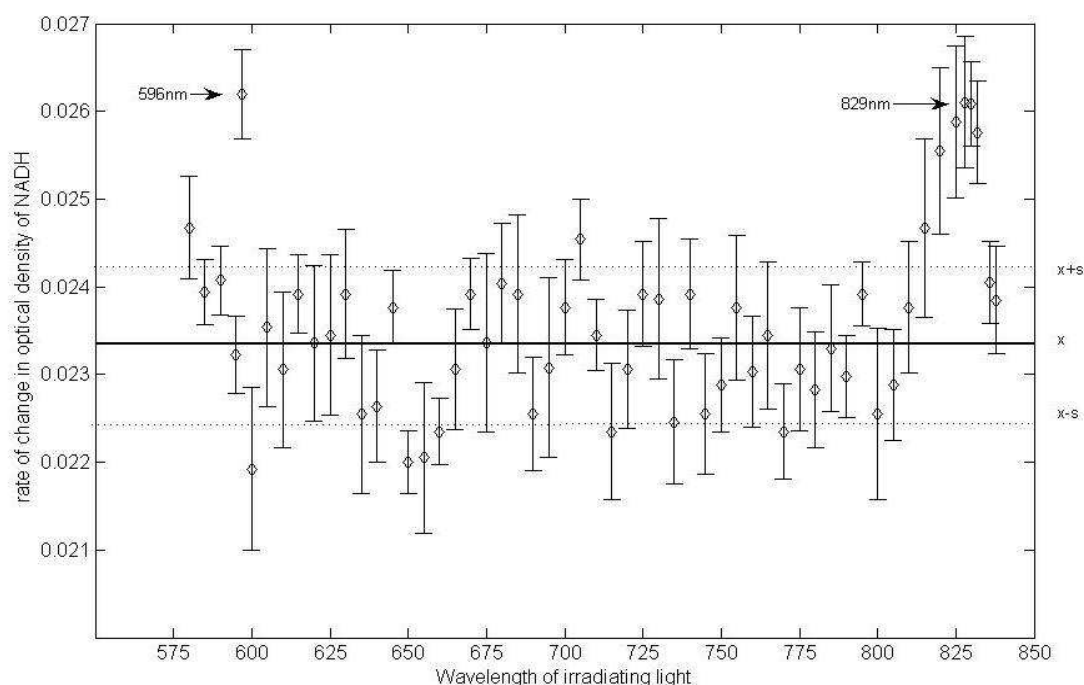
Fig. 2. The activity of the LDH is measured after irradiation of the LDH with different wavelengths of light.. The ordinate represents the rate of change in optical density of NADH per minute. By Beer-Lambert low the optical density is proportional to the concentration of absorbing molecule or NADH. Thus, the rate of change represents practically the rate of change of the NADH concentration per unit of time, or activity of the LDH. Horizontal bold line represents an average (x) for the non-irradiated samples. Horizontal dashed lines (x+s; x-s) values distant for one standard deviation (s) up and bellow from average value

The experiments were performed at room temperature 27°C (Temperature controller Quantum Northwest). The cuvettes were filled with 0.3ml of the LDH samples. The samples were previously irradiated with the light of different wavelengths (1140-1200 nm) for 600 sec. These irradiated samples were added then to the already prepared solution of NADH and pyruvate. The optical density of NADH was measured at 340nm foe each irradiating wavelength. The results obtained have revealed the change of the NADH absorbance under the influence of irradiated LDH.

From Fig. 3, we can observe that maximum optical density of the NADH is achieved at the wavelengths **1192 and 1200 nm** ($f_1=0.1688\pm0.004$) as was predicted by the RRM as the possible activation frequency of the dehydrogenase enzymes. Hence, the results suggest that this specific biological process can be modulated by irradiation with defined frequencies strongly supporting the main concept of the RRM methodology. The possibility to computationally calculate the frequencies with the following use of IR and visible light to produce the desired biological mutations and alterations in proteins would benefit the development of new biomaterials, non-invasive treatments and advanced technologies.
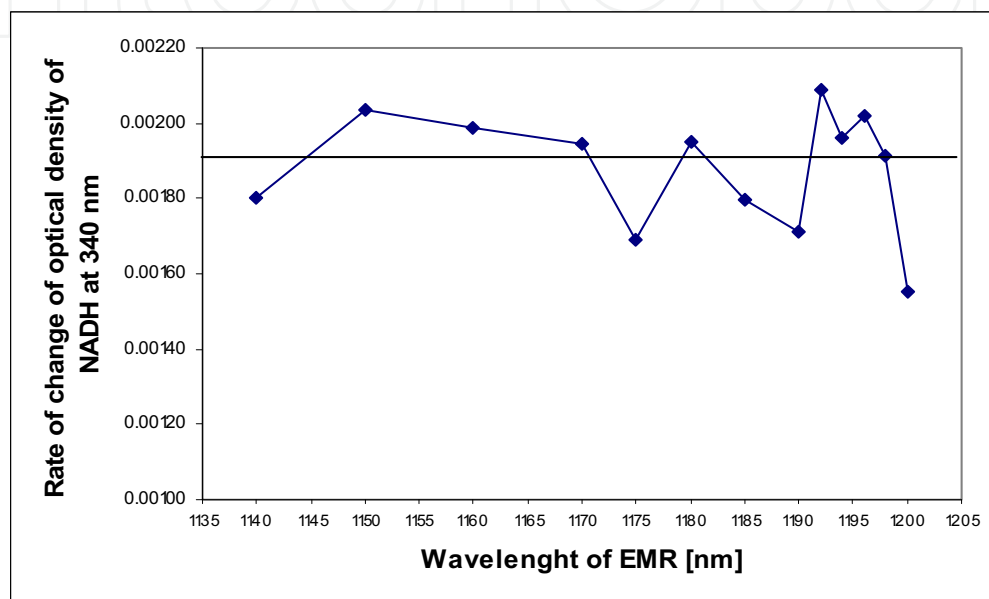
Fig. 3 Gradient of change in the NADH absorbance

4.3 Low level microwave radiation

In this study, the LDH samples were exposed to low level RF fields in the range of 400 MHz–1000 MHz. As a source of microwave radiation we will use a TC-5062A UHF TEM Cell (100 kHz – 3GHz), TESCOM Ltd. Through the input port, an external signal is applied to generate a predictable field inside the TEM Cell. A GTEM camera will be used to expose the enzyme solution samples. The GTEM can be calibrated using a broadband electric field probe to determine the electric field produced at the sample position inside the GTEM for a given input power. The field values provided for 10 dBm (10 mW) input to the power levels applied can be scaled using the following equation (TC-5060 Operating Manual):

$$E_1 = E_0 \sqrt{\frac{P_1}{10mW}} \tag{1}$$

Where E_1 is the exposure field of the sample, E_0 is the calibration field we found using a test power of 10 mW, and P_1 is the test power we used in our exposure.

The proposed exposure technique enables us to maintain the control (non-radiated) and exposed enzymes at similar temperatures, and to accurately monitor the specific absorption rate (SAR, mW/g) and temperature of the studied aqueous enzyme solutions. The estimated uncertainty in the generated field is ± 30%.

Procedure and data collection

1. To measure the absorption coefficients of the studied enzyme samples we used an Ocean Optics USB2000 spectrometer. Sampling rate for absorption coefficient at 340 nm was set at 1 sec.
2. The experimental solutions were prepared according to the assay description provided by Sigma-Aldrich.
3. The temperature was controlled by the Temperature controller (Quantum Northwest).
4. The enzyme solutions were exposed/sham exposed to ultra high frequency (UHF) electromagnetic radiation. The electromagnetic field (EMF) is generated by the certified Transversal Electromagnetic mode GTEM-cell TC 5061A with a signal generator, ROHDE&SCHWARZ Signal Generator SMX. The EMF inside of the test volume is proportional to the input voltage and inversely proportional to the cell height. Therefore, the electrical field generated at the test point inside the TC-5061A can be calculated as (TC-5060 Operating Manual):

$$E = \frac{signal\ level\ [V]}{dis.\ from\ top\ [M]} \tag{2}$$

Magnetic field produces by the GTEM-cell is orthogonal and proportional to electric field. Their relation is described by (TC-5060 Operating Manual):

$$magnetic\quad field(A/m) = \frac{Electric\quad field(V/m)}{377(Ohm)} \tag{3}$$

Although, we cannot distinguish which field is more responsible for non–thermal effects, we suggest that one of the main effects, i.e. change in protein's hydration is induced by applied electrical field.

5. The cuvettes were filled with the sample and kept at 22 cm distance from the top of the camera. The exposure duration was 240 sec.
6. After exposure the optical density of the NADH was recorded continuously for the next 5 minutes with the step of 1 sec. The control cuvettes were also kept under the same experimental conditions inside the camera with the signal generator being in the switched off mode (sham exposed).
7. The activity of the enzyme samples was calculated from the absorption curves using the gradient methods.
8. The frequencies of the applied radiofrequency irradiation were in the range of 400 MHz up to 1800 MHz with step of 25 MHz.

9. For each frequency of radiation the effect was measured for the following electrical fields: 0.02135 V/m, 0.06754 V/m 0.21358 V/m 0.675 V/m and 2.136 V/m (0.0000012 W/m², 0.000012 W/m², 0.00012 W/m², 0.0012 W/m², and 0.012 W/m² respectively).

10. The measurements were repeated 3-8 times depending on their standard deviations, irradiating frequency and electrical field values.

Data analysis

The data obtained were collected and analysed. The significance of the data was analysed using a single factor ANOVA analysis. For each frequency and power we tested H_0 hypothesis that variability among the irradiated and non-irradiated samples represents a random error. For the hypothesis being tested we set $\alpha = 0.05$.

The results obtained provide sufficient data for summarising on the influence of applied non-thermal radiation on the studied biological process. In particular, the results showed that biological activity of the LDH enzyme increased by 5%-10% using low power microwaves at 500-525 MHz for electric fields of 0.021-2.14 V/m. We also showed that 900 MHz irradiation increased LDH bioactivity for electric fields in the range of 0.021 V/m-0.068 V/m and decreased activity if the field was stronger than 0.67 V/m. We observed a slight inhibiting effect on LDH activity at the frequencies of 650 MHz (0.214 V/m); 700 MHz (0.68-2.14 V/m) and for 875-925 MHz (0.68-2.14 V/m), when LDH activity was decreased by 2%-15%. In particular, we observed the maximal increase in LDH activity, induced by the microwave radiation, at two particular frequencies (Figure 4).

In summary, biological activity increased and decreased as follows:

- increased - at 500 MHz and electric field of 0.021 (p< 0.17), 0.068 (p< 0.056), 0.213 (p< 0.038), 0.675V/m (p< 0.023), 2.14V/m (p< 0.021) (Figure 4).
- increased - at 900 MHz and electric field ranging from 0.021V/m (p<0.032) to 0.0675V/m (p<0.024) (Figure 4).

The outcomes of this study show that low intensity level microwaves of particular frequencies can induce changes in enzyme bioactivity and thus, affect the specific biological process involving the LDH enzyme. This important finding requires more comprehensive investigation with different enzyme examples and a wider frequency range (not just frequencies emitted by common RF devices) in order to better understand the effects of non-thermal RF radiation on proteins. The outcomes of this study will eliminate uncertainties in the present state of knowledge on the effects of radiofrequency radiation which are of concern to national and international regulatory and other health agencies.
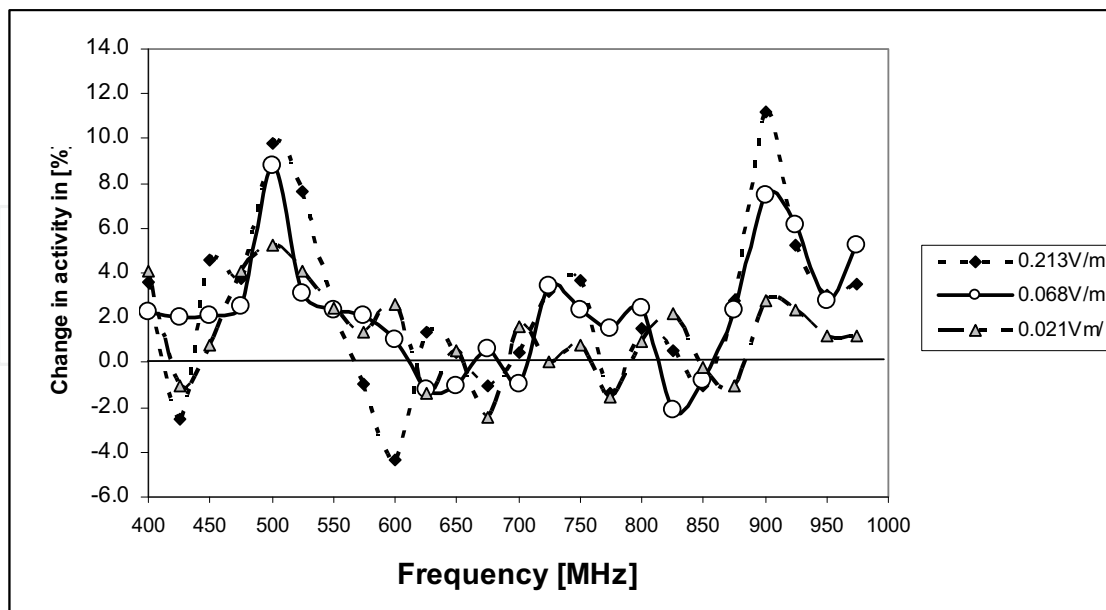
Fig. 4. Radiofrequency effects on LDH activity, calculated as relative rate of change of optical density of NADH in an irradiated sample to the rate of change in a non-irradiated sample, measured for electrical fields of 0.021V/m, 0.068V/m and 0.213V/m.

5. Conclusion

Our research presents an attempt to shed a new light on possible deeper physical grounds underlying the process of protein interactions. To elucidate the possible mechanism of influence of the EMF on proteins we have conducted a series of *in vitro* experiments where L-Lactate dehydrogenase was irradiated by visible light of different wavelengths ranging from 550 nm up to 900 nm, infrared exposures at 1140-1200 nm. These ranges of applied EMR were determined by the RRM. The computational results revealed that the RRM frequencies corresponding to the dehydrogenase enzymes activation are at $f_1=0.1688\pm0.004$ and less prominent at $f_2=0.2392\pm0.004$ (corresponding to 1191 ± 15 nm and 846 ± 15 nm respectively) can be directly related to the resonances in electron differential scattering cross section of these macromolecules.

The results obtained have shown the sensitivity of the reaction rate depends on the frequency of the visible light, albeit a low-intensity visible light has energy incomparable to the characteristics energies of electron excitations. Our experimental results are in a close agreement with the characteristic wavelengths corresponding to the activation of LDH enzyme predicted computationally within the RRM. Based on similarity between the RRM spectra calculated and the LDH activity spectra obtained experimentally we conclude that the distribution of the electron-ion potentials along the whole peptide chain could have a crucial impact on the interactive process between visible light and enzymes. The presented methodology may allow the generalization of the main advantage of the RRM in the case when the space structure of macromolecules is taken into account in a more realistic way. Based on the RRM spectral characteristic we can calculate the wavelength of the electromagnetic energy that can be used to modulate protein activity, hence giving rise to an

innovative efficient methodology to program, predict, design and modify proteins and their bioactivity.

In addition, we studied the effects of low level microwaves ranging from 400 MHz up to 975 MHz on the L-Lactate Dehydrogenase enzymatic activity. It was shown that extremely low level ultra high frequency irradiation with the electric field strength of 5-0.05 V/m can alter enzyme activity. The results of this study revealed that the biological function of the studied LDH enzyme can be increased by 10% using the low power microwaves at 500 MHz (0.02 V/m-2.1 V/m) and 900 MHz (0.021-0.21 V/m). Based on these results we can conclude that enzyme (and possibly other proteins) activity can be modulated by the specific frequencies of low power microwave radiation.

This finding may explain some non-thermal effect of microwaves on biomolecules and lead to a whole range of possibilities of controlled modulation of protein activity, which could benefit the development of new biomaterials, non-invasive treatments and advanced technologies. This could have major implications in drug design, medicine, agriculture, pharmacology and biotechnology. This finding could contribute towards development of a novel microwave technology for cancer diagnosis/therapy, treatment of metabolic diseases, and bacterial infections.

6. References

Ahme, M., Cashmore A.R. (1993). HY4 Gene of A. Thaliana Encodes a Protein with Characteristics of Blue-light Photoreceptor, *Nature*, 366:162-166.

Al-Watban, F.A.H. & Andres, B.L. (2003). Polychromatic LED Therapy in Burn Healing of Non-diabetic and Diabetic Rats, *Journal of Clinical Laser Medicine & Surgery*, V. 21(5): 249-258.

ARPANSA Australian Radiation Protection and Nuclear Safety Agency (in press) (2008) "Maximum Exposure Levels to Radiofrequency Fields – 3 kHz to 300 GHz, Annex 3: Epidemiological studies of exposure to radiofrequencies and human health, Sydney.

Biscar, G. (1976). Photon Enzyme Activation, *Bull. Math. Biology*, 38, pp. 29-38, 1976.

Breckenkamp, J., Berg, G., Blettner, M. (2003). Biological effects on human health due to radiofrequency/microwave exposure: a synopsis of cohort studies, *Radiat Environ Biophys*, Vol. 42, pp.141–154.

Byus, C.V., Lundak, R. L., Fletche, R. M., Adey, W. R. (1984). Altenation in Protein kinease activity following exposure of cultured human lymphocites to modulated microwave fields, *Bioelectromagnetics*, vol 5, no 3, pp. 341-351.

Cosic, I. (1994). Macromolecular Bioactivity: Is it Resonant Interaction between Macromolecules?-Theory and Applications,. on IEEE Transactions *Biomedical Engineering*, 41:1101-1114.

Cosic, I., Hearn, M.T.W. (1991). Hot spot amino acid distribution in Ha-ras oncogene product p21: Relationship to Guanine binding site. *Journal of Molecular Recognition*, vol. 4, pp 57-62

Cosic, I. & Hearn, M.T.W. (1992). Studies on protein-DNA interactions using the resonant recognition model: Application to repressors and transforming proteins. *Eur. J. Biochem*, 205, pp.613-619

Cosic, I. & Birch, S. (1994). Photoreceptors Having Similar Structure but Different Absorptions Can be Distinguished using the Resonant Recognition Model, Proceeding IEEE EMBS , 16: 265-266.

Cosic, I, Vojisavljevic, V. Pavlovic, M. 1989. The Relationship of the Resonant Recognition Model to effects of Low-intensity Light on Cell Growth, *International Journal of Radiation Biology*, 56: 179-191.

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

Dutta, S.K., Das, K., Ghosh, B., Blackman, C.F. (1992). Dose dependence of acetylcholinesterase activity in neuroblastoma cells exposed to modulated radio-frequency electromagnetic radiation, *Bioelectromagnetics* 13:317-322

Eberhardt, J.L. Persson, B.R. Brun, A.E. Salford, L.G. Malmgren L.O. (2008). Blood-brain barrier permeability and nerve cell damage in rat brain 14 and 28 days after exposure to microwaves from GSM mobile phones, *Electromagn Biol Med.*, Vol. 27(3), pp. 215-29.

Hardell, L. & Sage, C. (2008). Biological effects from electromagnetic field exposure and public exposure standards, *Biomedicine and Pharmacotherapy*, Vol. 62, Issue 2, pp. 104-109.

Fedoseyeva, G.E., Karu, T.I., Lyapunova, T.S., Pomoshnikova, N.A., Meissel, M.N. (1988). The Activation of Yeast Metabolism with He-Ne Laser Radiations-II. Activity of Enzymes of Oxidative and Phosphorous Metabolism, *Lassers in the Life Sciences*, 2(2):147-154.

Fesenko, E.E., Geletyuk, V.I., Kazachenko, V.N., Chemeris, N.K. (1995). Preliminary microwave irradiation of water solutions changes their channel-modifying activity, *FEBS Lett*. 366, pp. 49-52,.

Frochlich, H. (1984). IEEE Trans Microwave Theory Technology, Vol. 26, pp. 613-617.

Frohlich, H. (1986). Coherent excitation in active biological systems, *ModernBioelectrochemistry*, F. Gutmann, & H. Keyzer, eds., New York: Plenum, pp. 241-261.

ICNIRP Guidelines for limiting exposure to time varying electric, magnetic and electromagnetic fields (up to 300 GHz), *Health Physics* 1998, Vol. 74, pp. 494-522.

Jauchem, J.R. (2008) Effects of low-level radio-frequency (3 kHz to 300 GHz) energy on human cardiovascular, reproductive, immune, and other systems: A review of the recent literature, *Int. Journal of Hygiene and Environmental Health*, Vol. 211, Issue 1-2, pp. 1-29

Kappe, C.O. & Stadler, A. (2005). *Microwaves in Organic and Medical Chemistry*, Wiley-VCH, Vol. 25, pp. 16-25.

Karu, T. (1987). Photobiological fundamentals of low-power laser therapy, *IEEE Journal of Quantum Electronics*, QE-23: 1703-1717.

Karu, T. (1999). Primary and Secondary Mechanisms of Actions of Visible to Near-IR Radiation on Cells, *Photochemistry and Photobiology,* 49:1-17.

Karu. T.I., Pyatibrat, L.V., Kalendo, G.S. (2004). Photobiological modulation of cell attachment via cytochrome c oxidaze, *Photochemical and Photobiological Science*. 3(2): 211-216.

Kujawa, J., Zavodnik, L.; Zavodnik, I., (2003). Low-Intensity Near-Infrared Laser Radiation-Induced Changes of Acetylcholinesterase Activity of Human Erythrocytes. Journal of *Clinical Laser Medicine Surgery*. 21 (6):351–355.

Kundi, M. (2009). The Controversy about a Possible Relationship between Mobile Phone Use and Cancer. *Environmental health perspectives* 117(3):316-24

Nussbaum, E.L., Lilge, L., Mazzulli, T. (2002). Effects of 630-, 660-, 810-, and 905-nm laser irradiation delivering radiant exposure of 1-50 J/cm2 on three species of bacteria in vitro, *Journal of clinical laser medicine & surgery*, 20(6), pp. 325-33.

Pavicic, I. & Trosic, I. (2008). Impact of 864 MHz or 935 MHz radiofrequency microwave radiation on the basic growth parameters of V79 cell line *Acta Biol Hung*. 59(1):67-76.

Peinnequin, A., Piriou, A., Mathieu, J., Dabouis, V., Sebbah, C., Malabiau, R., Debouzy, J.C. (2000). Non-thermal effects of continuous 2.45 GHz microwave on Fas-induced apoptosis in human Jurkat T-cell line, *Bioelectrochemistry* 51, 157-161 .

Pirogova, E. Cosic, I. Vojisavljevic, V. Fang, (2008). Use of infrared and visible light radiation as modulator of protein activity, *Estonian Journal of Engineering*, Vol. 14, No. 2, 107-123.

Porcellia, M., Cacciapuoti, G., Fusco, S., Massa, R., D'Ambrosio, G., Bertoldo, V., De Resa, M., Zappia, V., (1997). Non-thermal effects of microwaves on proteins: thermophilic enzymes as model system, *FEBS* letters , vol. 402, pp. 102-106,

Sheppard, A.R., Swicord, M.L., Balzano Q. (2008). Quantitative evaluations of mechanisms of radiofrequency interactions with biological molecules and processes, *Health Phys*.; Vol. 95(4), pp. 365-96.

Stiller, M.J., Pak, G.H. Shupak, J.L., Thaler S., Kenny, C., Jondreau, L. (2006). A portable pulsed electromagnetic field (PEMF) device to enhance healing of recalcitrant venous ulcers: a double-blind, placebo-controlled clinical trial, *British Journal of Dermatology*, Vol, 127 Issue 2, pp.147-154

TC-5060 UHF TEM Cell Operating Manuel Tescom Co, Pty.

Tepper, O.M., Callaghan, J., Chang, E.I., Galiano, R.D., Bhatt, K.A., Baharestani, S., Gan J., Simon, B., Hopper, R.A., Levine J.P., Gurtner, G.C. (2004). Electromagnetic fields increase in vitro and in vivo angiogenesis through endothelial release of FGF-2 *The FASEB Journal*. 2004;18:1231-1233

Tian-Yong Zhao, Shi-Ping Zou, Pamela E. Knapp, (2007). Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes, *Neuroscience Letters*, 412, 34–38.

Toida M; Watanabe F; Kazumi Goto K; Shibata T. 2005. Usefulness of Low-Level Laser for Control of Painful Stomatitis in Patients with Hand-Foot-and-Mouth Disease, *Journal of Clinical Laser Medicine&Surgery*. 21 (6): 363–367.

Vojisavljevic, V., Pirogova, E., Cosic, I. (2007). The effect of Electromagnetic Radiation (550nm-850nm) on l-Lactate Dehydrogenase Kinetics, *Int. Journal of Radiation Biology*, Vol. 83(4), pp. 221-230.

Vorst, V., Rosen, A., Kotsuka. (2006). *RF/Microwave Interaction with Biological Tissue*. Wiley-Interscience, pp. 90-105.

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Edited by Carlos Alexandre Barros de Mello

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