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1 Introduction

Despite advances in material and biological sciences, many properties of biological structures remain unknown; partially due to lack of adequate models for interpretation of experimental data, partially because of experimental limitations. One example of poorly explored material property of biological structures is, especially at the nano-scale, represented by mechano-electrical coupling [1].

Although the coupling between mechanical and electrical phenomena is the crucial part of functionality of many biological structures and processes [1], it is not sufficiently understood yet. Moreover, this coupling was only rarely studied, to our knowledge, in connection with oscillatory processes, which are quite common to biological systems. The idea developed in this thesis is following: Majority of supramolecular structures of biological origin are electrically polar. Since these structures are under physiological conditions exposed to vibrational load, they should also vibrate to some extend. The charge bound to their structure will vibrate too. Vibrations of electric charge will generate oscillating electric field, role of which in cellular processes is discussed in this thesis. This process is also studied inversely, so the influence of external electromagnetic field on the vibrations of supramolecular structures is considered. These ideas are demonstrated on mictrotubules, supramolecular structures of cellular skeleton.

It is shown in this thesis, that mechano-electrical vibrations of microtubules may play role in sub-cellular morhogenesis and they may be utilized in diagnostics of cancer. Detailed computational simulations of this phenomenon are provided and technical aspects of measurement of electrical part of these vibrations are discussed.

2 Goals of the thesis

This thesis has the following goals:

- to review spontaneous mechanical oscillations in cells;
- to consider spontaneous mechanical oscillations of electrically polar supramolecular structures in cells as a generating mechanism of oscillating electric and electromagnetic field;
- to hypothesize about role of these oscillations in cellular physiology and morphogenesis;
- to analyze possibility of experimental verification of generation of electromagnetic field by cells in radio-frequency band;
- to consider medical applicability of mechano-electrical phenomena in cells, namely for diagnostics of cancer.

3 State of the art

3.1 Spontaneous mechanical oscillations in cells

In following, we will review spontaneous mechanical oscillations using bottom-up approach, i.e. starting at the level of chemical reactions and continuing up to the scale of whole cells.

3.1.1 Quantum effects and long-range correlations

The most influential concept describing generation of mechanical oscillations as a consequence of quantum effects is probably the Fröhlich's hypothesis [2, 3]. In a nutshell, it says that the energy supplied to the biological system can condense in (coherent) modes of longitudinal vibrations as a result of nonlinear interaction between elastic and polarisation fields. Although this hypothesis is far from general acceptance, even highly sceptical authors admit that energy condensation (at least weak condensate) is biologically feasible to a certain extend [4]. Attempts to reconsider Fröhlich's hypothesis have emerged recently [5,6].

There is also freshly opened question of quantum-coherent coupling of micromechanical oscillators to electromagnetic mode of a cavity [7] which may involve frequency transformation [8]. But its relevance for biological systems was so far not discussed.

3.1.2 Acoustical emission of chemo-physical processes

It was experimentally shown that chemical events may emit acoustical energy in a wide range of frequencies. Firstly, acoustic emission can be connected with chemo-physical processes like bubble formation, foam rupture or hydrodynamics of the sample. Some of these processes may be occasionally audible under certain conditions. Nevertheless, the frequency band of reported experiments span from ones of Hz up to hundreds of kHz, therefore exceeding the range of human hearing. Secondly, even chemical reactions themselves may be acoustically active in the range of 50 kHz upwards and the emission is usually attributed to

phase transitions. However, the methodology of measurement of emitted power was not consistent among published reports, therefore not enabling quantitative comparison. Comprehensive review of this topic may be found in reference [9].

3.1.3 Eigen modes of proteins and supramolecular structures

On the range of molecules, vibration dynamics of proteins is well developed discipline [10]. From the mechanical point of view, molecule may be seen as a discontinuous elastic body which may vibrate on its natural frequencies. Compared to smaller and simpler molecules, proteins have relatively low frequency of vibrations, typically in the region of THz.[1] This is because of their structure which involves a large number of atoms (which means higher mass) many of which are connected with weak bonds (e.g. hydrogen bonds) responsible for specific spatial conformation. The frequency of vibrations therefore enables investigation by means of THz and infrared spectroscopy [11]. For review see [12,13] and citations therein.

When proteins are bound together in supramolecules and supramolecular networks, the vibrational frequency of whole structure is accordingly lower. Based on nanomechanical measurement of elastic properties, calculations indicate that vibrations of filamentous supramolecular structures may lay in the kHz to GHz region [14, 15]. But the issue of damping becomes more important on these scales. Some authors presume that vibrations are overdamped due to viscous damping by cytosol [16] while others argue that the interfacial effects caused by electrical polarity of proteins may occur, making spontaneous vibrations physically plausible [17, 18]. However, direct experimental evidence is still missing.

3.1.4 Oscillations driven by molecular motors

Besides oscillations generated by accumulation of energy on the natural frequencies of subcellular structures there are also active mechanisms within the cell which may also generate spontaneous mechanical oscillations in the band between fractions of Hz and kHz range [19].

The active generation of mechanical force is connected with molecular motors. Among others, three families of cytoskeletal motors, which are present in eukaryote cell, play important role in cell's motility: ki-

[1] It is important to note that on such high frequencies quantum concepts involving phonons are more suitable for description of vibrations.

nesins, dyneins (both interacting with microtubules), and myosins (interacting with actin filaments). While single motor is simply walking along microtubule or actin filament, ensembles of motors may exhibit oscillative behaviour [20].

While single motors are used for intracellular transport (cargo is attached to the one end of the motor and the other end of the motor is attached to microtubule or microfilament), collective behaviour of motor ensembles is physiologically employed for motility of higher structures, or even whole cells. Oscillatory motility of undulipodia (motile cilia and eukaryotic flagella) is based on the activity of motor-proteins ensembles contained in axoneme (a structure arranged from microtubules). Oscillations of auditory hair bundle are associated with motor proteins too (myosins in this case), but it also involves feedback loops provided by mechanosensitive ion channels. Further reading may be found in review by [21].

3.1.5 Vibrations of whole cells

Vibrations may also occur on the level of cell wall, cell membrane or even on the scale of whole cell; however, there is usually an underlying mechanism which feeds these vibrations. It may usually be normal mode oscillations, manifestation of vibrations of subcellular parts, activity of motor proteins [22] or a combination of these.

Theoretical frequencies of mechanical resonances of bacteria are spread in a wide range of higher kHz up to higher MHz [23]. This frequency band may be significantly broader for other types of cells if one considers variability of their size, shape, and mechanical properties. Exposure to ultrasound – sonication – is widely employed for destruction of cells mainly as a result of disruption of cell membrane or disassembly of subcellular components (see for example [24]) due to resonant accumulation of acoustical energy.

Experimental evidence of mechanical oscillations measured *in vivo* on the level of whole cells was reported starting at fractions of Hz (membrane undulations of red blood cells [25]) and extending to kHz range including oscillations of cardiomyocytes [26, 27], oscillations of yeast's cell wall [28], and otoacoustic emission of hair cells [29, 30]. Generally, authors attributed these oscillations to a combination of mechanical properties of cells and particular subcellular vibrational process mentioned above.

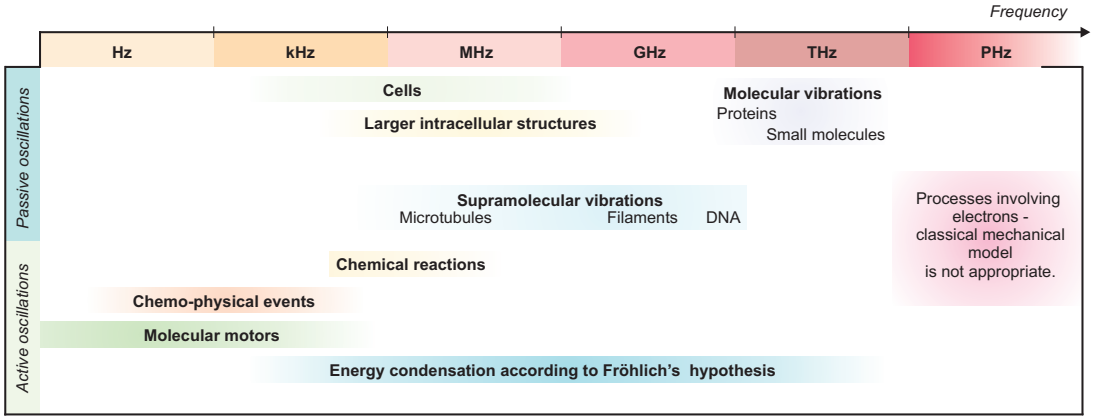

Figure 3.1: Schematic position of spontaneous mechanical oscillations in cells within the frequency spectrum. Passive and active oscillations are connected with the concept of linear and nonlinear oscillator, respectively.

3.2 Electrical oscillations in living matter

Existence of electric field is coupled to electric charge. Almost all proteins are electrically polar[2] and electrostatic properties of proteins oftentimes play essential role in their function. Interaction between proteins or protein–ligand interactions are often of electrostatic nature because they involve contact between charged or polar groups. Charge transfer or separation is usually involved in local chemical events in proteins [33]. Electrostatic interaction is also important for assembly of supramolecules (e.g. polymerisation of actin [34]) and electrostatic environment influences reactivity of their subunits (e.g. cysteine reactivity of tubulin [35]). On the higher level, electrostatics contribute to mechanics of tissues (e.g. compressive modulus of cartililage is strongly influenced by negative charges of proteoglycans [36]). For more details on electromechanical coupling in biomaterials see [1].

Below we proceed from electrostatics to oscillating electric field. Oscillating electric component of electromagnetic field may spring from oscillative motion of free or quasi-free electrons, ions, or bounded charge. It may be also radiated by excited electron during its fall to lower energetic level.

Oscillations of electric field caused by flow of ions through ion channels (which may be, among other mechanisms, driven by electric field or mechanical pressure) are well known basis of electrophysiology.

[2]Electric field changes vibrational spectrum of molecules (vibrational Stark effect) which helps to map internal electric field in proteins [31, 32].

There is also *in vivo* evidence for mechanical oscillations of a layer of ions separated by a membrane [37]. Quantum effects resulting in photon emission or excitation of electrons into conductive band were reported too [6, 17]; however, we will focus solely on oscillating electric field generated by oscillations of bound charge in living matter, particularly microtubules.

3.2.1 Mechano-electrical oscillations of supramolecular structures

For their electrical polarity, proteins are good candidates for mechano-electrical oscillations. As we have shown in Section 2, there are spontaneous mechanical oscillations of proteins, and probably also protein-composed structures. Moreover, there are other mechanisms described above which are most likely able to drive their vibrations. Therefore we consider it worth asking how the pattern of oscillating electric field will look like and what effect it may have.

Electromechanical coupling is poorly explored on the nanoscale, especially when talking about biomaterials [1], and mechano-electrical oscillations of supramolecular structures remain relatively unresearched. This fact may be attributed to doubts that arose about their feasibility *in vivo* and, more importantly, the experimental difficulty connected with attempts to prove their existence. To our knowledge, there is no direct experiment regarding mechanical and electrical oscillations of supramolecular structures measured simultaneously or even separately. So we have to rely on computations and deduce from what is known about mechanical, electrical, and energetic circumstances. In what follows, some simplifications will be therefore necessary. Most importantly, we will at first separate dynamics of a supramolecule and its electrical properties, although they are mutually connected because force interaction on the level of atoms is of electromagnetic nature. We find it advantageous for our argumentation, because the plenty of experimental work regarding mechanical oscillations in cells (although not regarding supramolecular structures) is in deep contrast with almost none reports regarding electrical oscillations, although they are coupled with the former.[3]

[3] Or, with less physical exactness but according to the way of our argumentation, they are implied by the former.

4 Methods and results

Microtubules (MTs) constitute important part of eukaryotic cytoskeleton. In contrast to the skeleton of a body, cytoskeleton is not only a mechanical support of the cell but also an active network responsible for self–organisation of a cell. MTs are present in a cell most frequently in the form of single cylindrical tubes (inner diameter 17 nm and outer diameter 25 nm) consisting of 13 protofilaments (see Fig. 4.1). More complicated shapes are also possible; however, the protofilament is their primary building block. Protofilament is assembled from alternating monomers of α-tubulin and β-tubulin (together forming heterodimer of tubulin). The number of MTs in a cell is strongly dependent upon the cell type and the phase of the cell cycle.

Microtubules play important role in physiology of eukaryote cells [38, 39]. Properties of microtubules' network are altered in number of pathological states of cells and microtubules are also targets for some therapeutic strategies (see e.g. refs. [40, 41]). Microtubules, as well as other supramolecular mesostructures, are also promising for future use in bio-electronic circuits [42].

What attracts attention to MTs, when talking about mechano-electrical vibrations, are their electrical and mechanical properties. Heterodimer

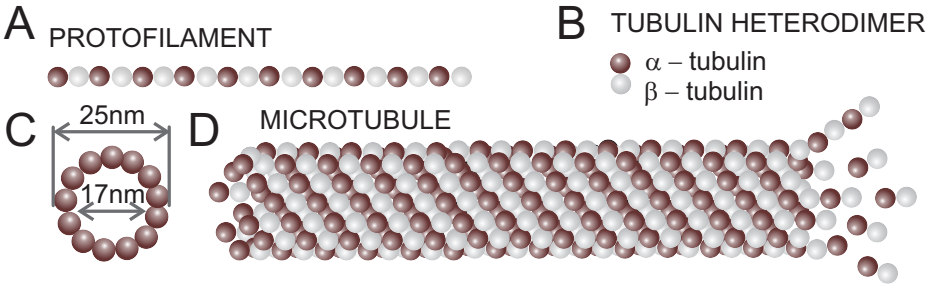

Figure 4.1: Schematic depiction of a microtubule, showing one protofilament (A), heterodimer of tubulin, its building block (B), cross-section of microtubule (C), and side view of one microtubule (D) which undergo dynamic instability on its right end.

of tubulin is strong electrical multipole with axial component constituing static dipole moment as strong as 1.12×10^{-27} C.m [43]. MT is therefore highly electrically polar structure. Mechanical properties of MT are extraordinarily anisotropic, which is the reason of extremely large variation of published values of its mechanical moduli [44].

4.1 Origin of MT's vibrations

There is a number of sources of energy which may drive vibrations of MTs. In their detailed analysis, Cifra et al. [45] suggest three types of energy sources. Firstly, the energy released from hydrolysis of GTP (guanosine triphosphate) during polymerisation of MT; secondly, the energy originating in the movement of motor proteins along MT; and thirdly, the "wasted energy" form mitochondria. Here we would like to add kind of fourth, indirect, source of vibrational energy from other sources mentioned in previous section, because this energy may be transmitted through mechanical environment of a cell to MTs.

If the process is linear, or quasi-linear, then MT may be viewed as linear oscillator with two possible regimes of operation. On the one hand, the system may be overdamped (quality factor, $Q < 0.5$) and dissipate energy without resonance. Then the amplitude of vibrations of MT will be lower or theoretically equal to the amplitude of the feeding mechanism. Frequencies of the feeding and MT vibrations will be equal. As a result, MT will behave like transmitter of external vibrations. We may expect that it will behave like a low-pass filter [46, 47]. This means that only frequencies between zero and so called cut-off frequency will be transmitted. On the second hand, the system may be underdamped (quality factor, $Q > 0.5$) and exhibit resonant behaviour. Then it would selectively accumulate energy on its resonance frequencies. The source of energy then does not necessarily need to have purely vibrational character, but it just need to have frequency spectrum wide enough to overlap with resonance frequency. MT will thus behave like resonator. As we already mentioned in before, the issue of damping is crucial for vibrations of supramolecules. Some authors [16, 48] argued that vibrations of supramolecular structures are overdamped by cytosol. This claim excludes resonant behaviour of MT; however, its function like an attenuating transmitter is not affected. If interfacial effects take place [17, 18], then vibrations may become underdamped.

In fact, MT is not object which may be represented by lumped element model of oscillator. It is a body with a number of vibrational modes. However, the analogy with oscillator is suitable for each mode

of vibrations. Many theoretical models of how does the MT transmit and accumulate vibrational energy were published. Here we do not go to details – interested readers may find detailed information in references [15, 49–57].

As may be seen in Fig. 3.1, vibrational energy exists in a cell in entire frequency spectrum. This energy may be transmitted over and/or accumulated in MT. Generally, the most efficient mechanism of generation of MT vibrations is that which involves resonance. In this particular case, the frequency of driving force is identical or very close to natural frequency of MT. Since MT has a large number of vibration modes and high variability of its length, the range of possible resonance frequencies is very broad. The problem becomes even more complicated if we consider protein complexes coupled to MT and boundary conditions generally. If we accept theoretical range of higher kHz to GHz as a real range of possible resonance modes of MT, then following sources have similar frequencies. Firstly coupling of passive oscillations of cells body and larger intracellular structures fits to this range. Partial frequency overlap is also achieved for vibrational emission from chemical reactions and entire range is covered by thermal noise and Fröhlich's hypothetical condensation. Possibility also exists that non-vibrational events with broad spectrum may provide energy for driving of natural modes. All other sources very probably do not allow resonant driving. Then the forced vibrations of MT are also possible, but energetic demands become more substantial. An example *par excellence* of such forced vibrations of MT are oscillations driven by motor proteins in axoneme.

However, if the process of generation of vibrations is nonlinear, then such a simple analysis is out of the question. In this case, the generating mechanism must be precisely identified. Otherwise, the variability of parameters to be chosen leads to a vast number of dramatically different solutions. Although some authors speculate about nonlinearity, this idea is almost unexplored with the exception of Fröhlich's condensation. In general words, vibrational energy may be then coupled between modes and different frequencies in such a way that almost each process mention in Fig. 3.1 will allow efficient driving of MT vibrations. Such a conclusion was also drawn in [45].

Needless to say, excitation of particular vibrational mode depends strongly on the spatial character of feeding mechanism. One may gain opposing results when considering local feeding or excitation by plane wave, etc. In our model below, we do not deal with any specific kind of generating mechanism. We simply assume that the vibrational energy,

which is present in the cell, was delivered to particular vibration mode. We are aware that this issue requires thorough analysis in the future, but now it appears to be outside of the scope of this manuscript.

4.2 Electric field generated by vibrations of MT

Mechanical vibrations of MT will be coupled with oscillating electric field. Pioneering estimate of the electric field generated by axial longitudinal vibrations of MT was published by Pokorný [50]. More accurate calculation was performed by Cifra et al. [58] using what we suggest to call the Microtubule Resonance Dipole-Network Approximation (MRDNA) method. This method was also used for simulation of power radiated from entire network of MTs [59].

Detailed description of MRDNA method is given in [58]. Here we only briefly summarise its principle and parameters used in calculations. Electric field of each tubulin heterodimer was approximated by elementary electric dipole. This dipole moment is the projection of total electric multipole moment of the tubulin heterodimer into the direction of vibrations. Since amplitude of vibrations is smaller than the length of the dipole, only a part of total dipole moment will contribute to oscillations. In our model we used one eighth of total dipole moment. Resulting oscillating dipole moment is modulated according to the local direction of vibrations of the MT. The electric field of whole MT or MTs' network is calculated as a vector summation of contributions from all heterodimers. The dipole–dipole interactions are not taken into account.

Here we use MRDNA method to demonstrate electric field which establishes between two vibrating MTs. The V-shape conformation of MTs (angle between their axes was 52°) was chosen in order to resemble two MTs growing from the centrosome. Results with different angle are provided in supplementary material. Two cases were studied. In the first case, both MTs had the same length of 156 heterodimers (1.248μm). In the second case, one of MTs was shorter (88 heterodimers = 0.624μm) than the other. Acoustic branch of axial longitudinal vibrations was investigated. Frequency of oscillations was 250 GHz which corresponds to mode number 10 for the longer MT and mode number 5 for the shorter one. Following parameters were used in our calculations. We have chosen MTs lattice B, which means that the shift between protofilaments in MT is 0.92 nm. Relative permittivity of the surrounding medium was $\varepsilon_r = 6.3626$, and conductivity was $\sigma = 72{,}52$ S/m [60]. The amplitude of vibrations was 0.1 nm. Concern-

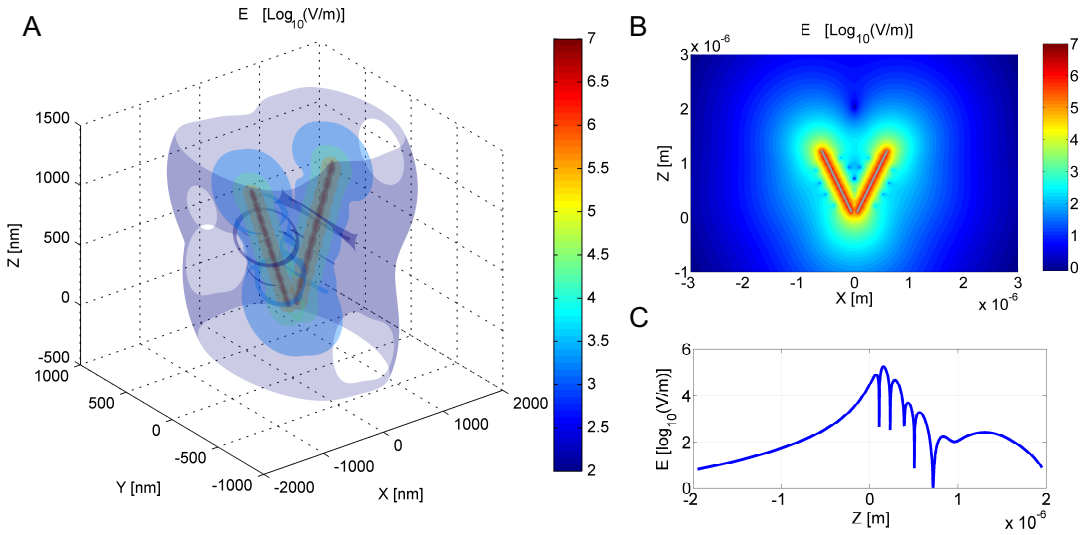

Figure 4.2: Results of the calculation of electric field generated by vibrations of two equally long MTs vibrating in phase. Isosurface of the intensity of electric field (A) evince local minima with the shape of a ring. Section through the field in the plane which contains axes of both MTs is shown in part (B) and (C) shows detailed shape of the intensity of electric field along vertical axis of section (B).

ing the phase of vibrations, we focused on two special cases. The case where vibrations of both MTs are in phase corresponds to common coherent feeding. The other case where the phase of vibrations is shifted for $\pi/2$ radians may be attributed to elastic coupling between MTs.

Results of the calculation are presented in Figs. 4.2 and 4.3. Fig. 4.2 shows the case of equally long MTs. It presents only time-lock of oscillating field. A movie may be found in supplementary material. Isosurface of intensity of electric field generated by equally long MTs is depicted in Fig. 4.2-A. It shows the contour of the field in the space. Section through the field in the plane containing axes of both MTs is presented in Fig. 4.2-B and detailed curve in the vertical axis of the conformation is shown in Fig. 4.2-C. The Fig. 4.3 is dedicated to the case when one MT is shorter. It shows different time-locks of the shape of the field in the plane containing axes of both MTs.

We observed local minima of the intensity of electric field. These minima have shape of a ring in the space (see Fig. 4.2-A). Minima change their position during the period of vibrations. We indicate this movement by arrows in Fig. 4.3-D.

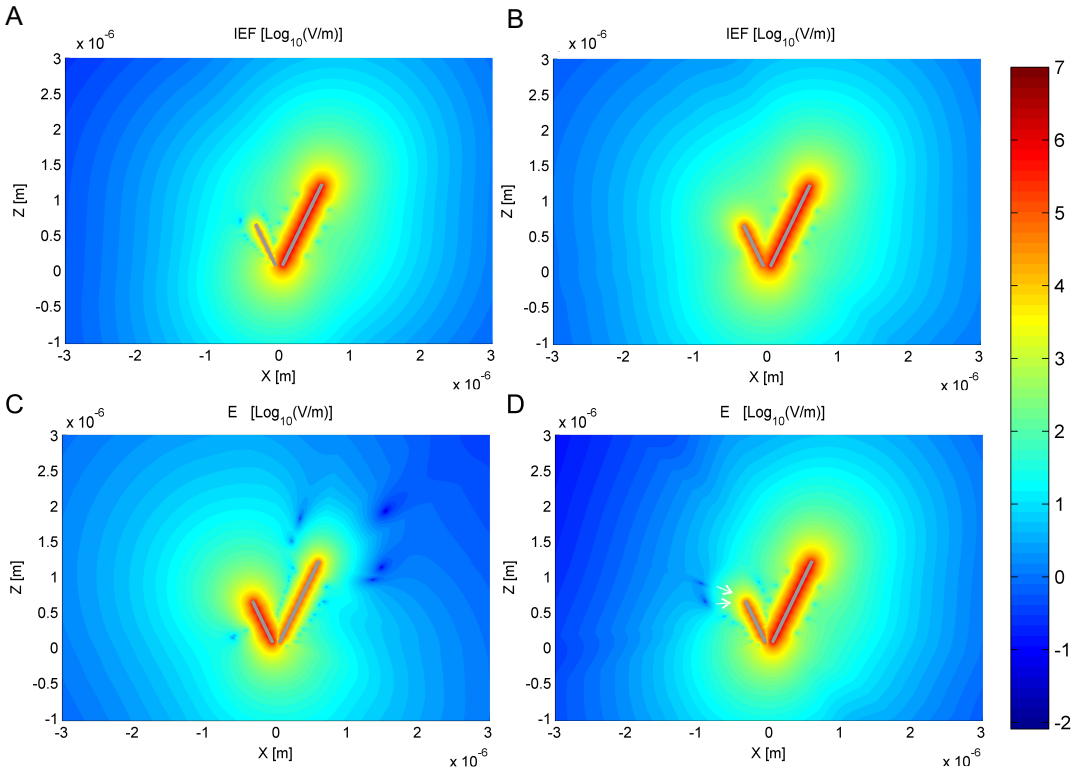

Figure 4.3: Electric field generated by $\pi/2$ radians shifted vibrations of two MTs of different length. Parts (A-D) depicts the intensity of electric field in the plane which contains axes of both MTs. Direction of movement of local minima is indicated by arrows in part (D). Time-locks were acquired in the phase of 358° (A), 18° (B), 276° (C), and 348° (D) of vibrations of the longer MT.

5 Discussion

5.1 Implications for experiments

The very first thought of results presented here naturally leads to the possibility of their experimental verification. As we have already mentioned, no experimental work unifying mechanical and electrical vibrations of supramolecules has been published yet. In the case of electrical oscillations, there have however been reports of resonant interaction of MTs with external oscillating electric field. This indicates the existence of oscillation states in MTs alone [6].

Some authors also tried to explain their experimental results obtained on the level of whole cells by vibrations of MTs. Pokorný [61] attributed discovered resonant interaction between nonlinear electromagnetic oscillator and tumours to vibrations of MTs. Pokorný [62] also found correlation between electromagnetic activity of yeast cells and formation of mitotic spindle during M phase of the cell cycle. Pelling et al. [63, 64] demonstrated a local oscillatory nanomechanical motion of the cell wall of yeast cells (frequency was about 1 kHz) and attributed it to concerted activity of motor proteins. Measurement of such oscillations was partially reproduced by Jelínek et al. [65], who afterwards attempted to measure electric field generated by these vibrations. Jelínek [65] concluded that there were significant differences of measured power between different phases of the cell cycle. Janča [66] analysed this data using neural networks and identified significant frequencies in electrical signal. However, the issue of sensitivity of the measurement system remains unsolved as mechanical and electrical signals were not measured simultaneously. Other results concerning electrical oscillations of living cells and non-thermal effects of electromagnetic irradiation may be attributed to mechano-electrical vibrations of MTs too [67, 68].

Technical aspects of measurement of electrical component of mechano-electrical oscillations in radio-frequency range were analysed by Kučera [69], but only on the level of whole cells. It was shown that accurate measurement must be done in the immediate vicinity of the cell using

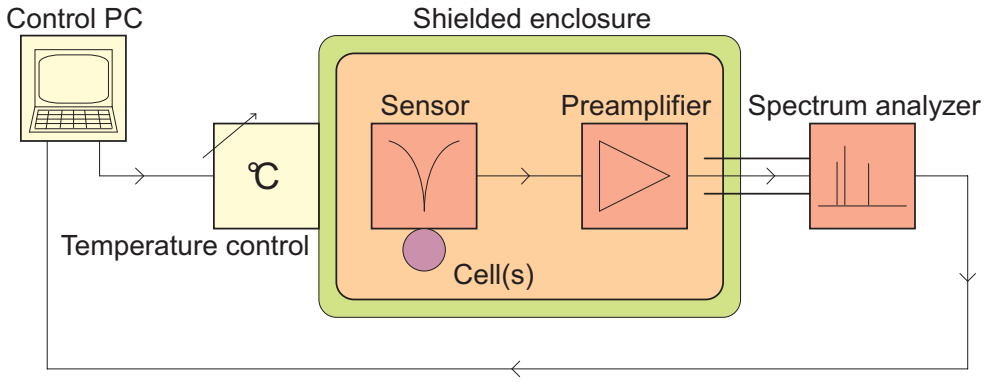

Figure 5.1: Block diagram of experimental setup for measurement of cellular electromagnetic activity.

a sensor making the low power signal from a soft nanoscale source accessible to the macroscopic measurement system. The device must be able to:

- Detect a signal of the order of magnitude of thermal noise.
- Achieve the spatial resolution required (hundreds of nm or better).
- Measure the near field component.
- Match the resistance and capacity of the nanoscale cellular source (high effective impedance for kHz and MHz frequencies).
- Shield itself from the various sources of noise.
- Form a transition link from nanoscale structures to macroscopic systems.

Limitations for mechanical measurements in range up to tens of kHz were discussed also by Gittes [70]. Combining these experimental prerequisites seems to be rather complicated, because we may expect spontaneous mechano-electrical oscillations to take place only *in vivo* due to presence of appropriate energy feeding[1] and coupling. Then the question is: How to bring the probe inside the cell? Although advances have been made in this direction [71–74], more realistic at the beginning seems to be measurement from the outside of a cell along recommendations presented in references [69, 70] (for instance by using some branch of scanning probe microscopy [1, 75, 76]) with the awareness of unspecificity arising from the high number of intracellular sources of oscillations. Calculations indicate [77] that electromagnetic power

[1] Remember that energy in chemical form constitute only minor part of possible feedings presented in this paper.

emitted from a cell as a result of vibrations of whole microtubular network is on the level of thermal noise; however, the magnitude of intensity of electric field is still sufficient for detection.

Mechano-electrical oscillations of MT *in vitro* may be obtained by driving of the MT by electric field and measuring the mechanical response and *vice versa*. But to our knowledge, no experiment in this direction has been published yet.

5.2 Implications for morphogenesis

Relevance of electric component of mechano-electrical oscillations of MTs for morphogenesis may be, according to our opinion, reduced to the question of force effects of electric field. These force effects may be employed for the purpose of information transfer or directed motion of the matter.

The role of endogenous electromagnetic forces in transfer of reaction components was discussed by Pokorný [78]. He distinguishes three different stages of chemical reactions: translation of reaction components to the region where the reaction takes place; short range motion of components in order to adjust appropriate space position; and the formation of chemical bonds. We will analyze this force effects on the case of tubulin dimer. The idea behind this choice is that oscillating electric field may govern transport of tubulin dimers towards the plus-end of growing MT.

The model we used has shown that directional transport of single tubulin dimers by generated electric field does not seem to be efficient under parameters we considered. The translational force, $\mathbf{F_d}$, acting on the electric dipole of free tubulin dimer (with moment $\mathbf{p} = 1.12 \times 10^{-27}$ C.m), $\mathbf{F}_d = \nabla(\mathbf{p} \cdot \mathbf{E})$, reaches its maximum in the order of about 10^{-19} N. Moreover, this maximum is tied together with the position of maximal gradient of intensity of electric field. As may be seen in Fig. 4.2-C, the local maximum of this gradient is located between neighbouring local minima and maxima of the intensity of electric field. As this position is dramatically changing during the period of vibrations, the effective force acting on the dipole is even lower. Truly feasible translational effect on tubulin dimer may be observed under these conditions only for resonant interaction. However, recent findings suggest that MT elongation and nucleation involves interactions of short tubulin oligomers rather than dimers [79]. The dipole moment is defined as $\mathbf{p} = q\mathbf{d}$, where $\mathbf{d}$ is the displacement vector (with the size of the length of the moment) and q is the charge. When dipole moments are arranged in a

line, then the neighbouring charges with opposite sign compensate each other. Only charges on the ends of the oligomer contribute to the resulting dipole moment and the total charge of the oligomer is then equal to the charge of a dimer. But what is changed is the length of the dipole, **d**, which is lengthened. The resulting force is then proportional to the number of dimers in the oligomer. As we may expect this number to be in the order of ones, then the force, $\mathbf{F}_d$, is still too small to exert efficient translational effect. However, this claim is based on the results of our calculations parameters of which may not entirely correspond to physiological reality. More optimistic results should be expected for different boundary conditions and spatial conformations. This direction should be therefore followed in the future research.

Translational effects may be also caused by dielectrophoretic force, i.e. the force acting on the polarisable dielectric particle in the inhomogeneous electric field. This force is proportional to the volume of the particle (V), the difference between permittivity of the particle (ε_p) and the medium (ε_m), and the gradient of the square of the intensity of electric field, so $\mathbf{F}_\varepsilon \approx sV(\varepsilon_p - \varepsilon_m)\nabla\mathbf{E}^2$. Besides the properties of the field and the particle, the force, $\mathbf{F}_\varepsilon$, is also governed by dielectric properties of the ambient medium. Very important parameter is also the shape of the particle, here expressed by the parameter s. In general words, the larger is the asymmetry of the shape of the particle, the larger is the value of s. Tubulin dimer or oligomer is extraordinarily inhomogeneous material and its dielectric properties were not satisfactorily documented so far. If we adopt elliptical homogenous model inspired by works on viruses [80, 81], we gain approximately identical results like in the previous case of force $\mathbf{F_d}$. As we mentioned therein, different parameters of the model and resonant interaction may lead to values of average force that is capable to overcome thermal fluctuations. Novel discoveries concerning Brownian motion [82] may also change our view on thermal fluctuations of the particle in the fluid. Influence of deterministic force on this particle can therefore be larger then in recent models involving Stokes drag.

Achievement of appropriate space position of components implies short range motion which is supposed to be of rotational or deformational character rather than translational nature. Electrorotational torque, $\tau = \mathbf{p} \times \mathbf{E}$, will act on the particle with dipole moment in order to align it with the external field. However, electrostatic interaction due to static dipole moment seems to be more efficient at this scale. Oscillating part of the field may, nevertheless, help with achieving of desired position.

The most promising therefore seems to be the influence of oscil-

lating electric field on the charge transfer. Pokorný [83, 84] analysed transport of electrons in the molecular chains outside the regions of redox potential under the presence of deterministic force. They conclude that electrons may be shifted to the target as far as 20 nm in the time scale under 1 ns (which corresponds to frequency of 1 GHz) with probability almost equal to 1 if the intensity of electric field is 10^6 V.m. Maximal values of the intensity of electric field in our calculations are of the same order and they are distributed in the vicinity of the MT. Profound effect of the electric field in regions far from MT is also possible, but it requires longer time to reach the same probability.

Information transport by means of oscillating electric field is possible only under the condition that there is a mechanism capable of detection of this field. Oscillating electric field generated by vibrations of MTs has, according to our model, relatively low magnitude. It is therefore evident, that the efficient and instant information transfer on a large distance (i.e. within a cell) would be possible only for the case of resonant interaction or electron transfer as a detection mechanism.

5.3 Implications for cancerogenesis

Cytoskeleton in cancer cells undergoes disintegration which results in disordered morphology and altered mechanical properties of cancer cells [85]. These changes are not only consequence of cancer process but also significant part of its patophysiology. There is extensive experimental evidence that mechanical properties of metastatic cancer cells significantly differ from that of healthy cells or even non-metastatic cancer cells and that the metastatic potential of a cell is well correlated with its stiffness. All these properties are essential for uncontrolled growth and metastatic proliferation.

From the point of view of mechanics, such a dramatic change in structure and stiffness must be also accompanied with change of dynamic properties, for example frequency of spontaneous oscillations or response to external vibrations.

Pokorný [86] developed theory which unifies mechano-electrical vibrations of microtubules with recently revisited role of mitochondrial dysfunction in cancer cells (Warburg effect). Dysfunction of mitochondria implies decline of the zone of the strong static electric field and of the space charge layer of protons around mitochondria. It was shown that this changes are followed by disruption of the level of water ordering. This, together with decreased efflux of the non utilized energy from mitochondria, changes damping of mechano-electrical vibra-

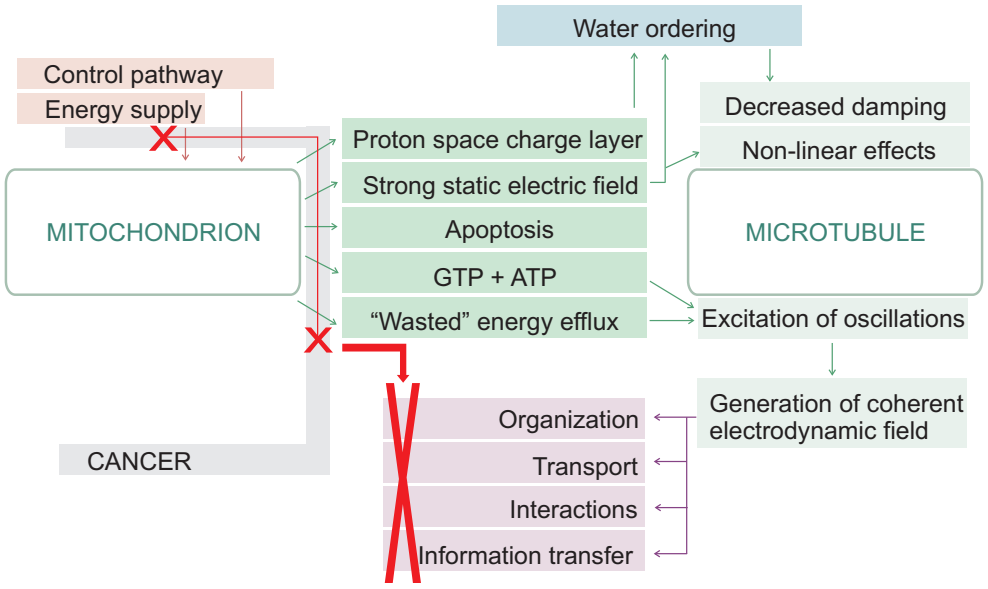

Figure 5.2: Physical links in the biological activity of living cells. A unique cooperation of mitochondria and microtubules is shown together with the areas of input controlling signals and output effects. Disruption of these links by cancer process is depicted in red colour.

tions of microtubules. This effect was employed in diagnostics [61] by TRIMprob^{TM} system.

Implications of this phenomenon for cancer transformation pathways and cancerogenesis may be following. Increased damping of microtubule vibrations will manifest itself in the drop of amplitude of spontaneous oscillations. The resulting oscillating electric field will be weaker and any effect of this field as well, so the contribution to organisation, information transfer and negative entropy production will be lower which well corresponds with disorganisation of cancer cells.

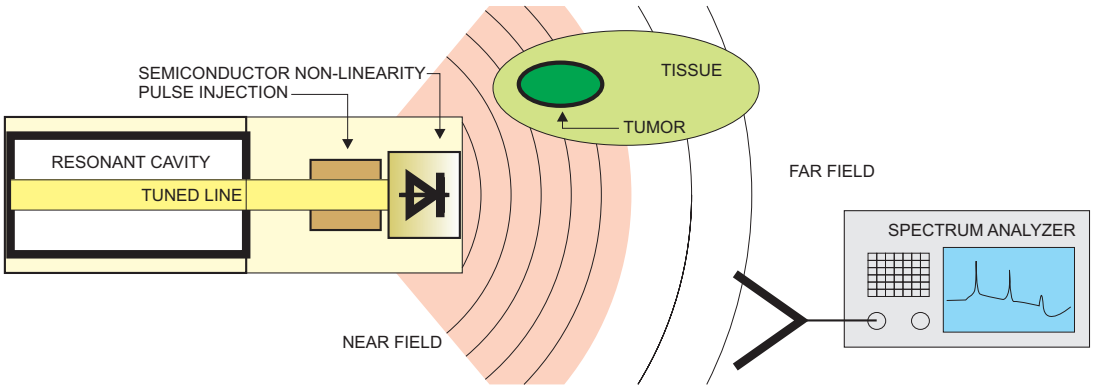

Figure 5.3: Schematic realization of the TRIMprob^{TM} system.

6 Conclusions

6.1 Contribution of the dissertation

This dissertation has provided novel theoretical connection between spontaneous mechanical oscillations in cells and electrical oscillations of electrically polar supramolecules, especially microtubules [A1]. Model of electromagnetic field, and namely its electrical component, generated by mechanically vibrating microbules has the highest precision published so far [A2]. Theoretical considerations of experimental verification of published models were firstly summarized here [A3]. Importance of mechano-electrical vibrations in microtubules for morphogenesis was disscussed [A1] together with implications for cancer diagnostics [A4].

6.2 Future directions

Since results presented in this dissertation [A1-A4] have, more or less, theoretical nature[1], future developments should provide experimental verification of these ideas. Two approaches to this problem may be found. Firstly on the level of cells, measurement of oscillating electric field in radio-frequency range should be performed. Although it may seem very straightforward, there are many technological challenges to be overcame for measurement *in vivo* as we have shown in paper [A3]. Secondly, the issue of experimental investigation on the level of supramolecular structures appears even more demanding. However, it is not necessary to measure *in vivo* on this level at the initial stage of research. One can therefore take advantage of modified experimental setup with substituting for example endogenous mechanical vibrations with technical feeding. Good experimental data will subsequently allow update of theoretical models and deliver deeper understanding of phenomena discussed in this thesis. Future research may in conclusion follow these directions:

[1] Experimental work on this topic is in progress and was not included in this dissertation.

- Development of nanotechnological sensors with preamplifiers and construction of auxiliary equipment for measurement of electrical component of electromagnetic field emitted by cells in radio-frequency range.
- Development of methodology for measurement of mechano-electrical vibrations of microtubules and other supramolecular structures; at the beginning *in vitro* and later under physiological conditions *in vivo*.
- Development of more detailed models of mechano-electrical vibrations of microtubules with precise estimate of the influence of hydration layer.
- Application of discovered mechanisms in medical diagnostics and artificial bio-electronic high-frequency circuits.

Each of this four directions implies specific scientific and technological challenges. Benefit from their overcoming would not be represented only by novel biophysical findings, but also in form of necessary technical innovations.

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List of author's publications related to the doctoral thesis

All authors contributed equally, unless otherwise states.

Papers in Impacted Journals

[A1] O. Kučera and D. Havelka, "Mechano-electrical vibrations of microtubules–Link to subcellular morphology," *BioSystems*, ARTICLE IN PRESS, 2012. Candidate's contribution: 65 %

[A2] D. Havelka, M. Cifra, O. Kučera, J. Pokorný, and J. Vrba, "High-frequency electric field and radiation characteristics of cellular microtubule network," *Journal of Theoretical Biology*, no. 286, pp. 31–40, 2011. Candidate's contribution: 12.5 %

[A3] O. Kučera, M. Cifra, and J. Pokorný, "Technical aspects of measurement of cellular electromagnetic activity," *European Biophysics Journal*, vol. 39, no. 10, pp. 1465–1470, 2010. Candidate's contribution: 80 %

[A4] J. Pokorný, C. Vedruccio, M. Cifra, and O. Kučera, "Cancer physics: diagnostics based on damped cellular elastoelectrical vibrations in microtubules," *European Biophysics Journal*, vol. 40, no. 6, pp. 747–759, 2011.

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Patents

We have no patents related to the doctoral thesis.

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[D1] M. Cifra, J. Pokorný, F. Jelínek, and O. Kučera, "Vibrations of Electrically Polar Structures in Biosystems Give Rise to Electromagnetic Field: Theories and Experiments," in *PIERS Proceedings*. Cambridge, MA: The Electromagnetics Academy, 2009, pp. 138–142.

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Grant No. SGS10/179/OHK3/2T/13 *Cellular Nano-Electromechanics*, Grant Agency of the Czech Technical University in Prague, 2010-2011, Principal investigator.

Grant No. GD102/08/H008, Czech Science Foundation - GACR, 2010-2011, Member of the researching team.

Grant No. P102/11/0649, Czech Science Foundation - GACR, 2011-2012, Member of the researching team.

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All authors contributed equally, unless otherwise states.

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Résumé

Dizertační práce se zabývá mechano-elektrickou vazbou v mikrotubulech, supramolekulárních strukturách buněčného skeletu. Tato mechano-elektrická vazba je studována především ve vztahu k vibracím mikrotubulu, během nichž je elastická deformace doprovázena tvorbou oscilujícího elektrického pole. Na základě detailních výpočetních modelů je analyzována možnost měření elektrické složky těchto vibrací a je podpořen nanotechnologický přístup k této otázce. Dále je diskutována možnost, že mechano-elektrické vibrace mikrotubulů hrají roli ve vnitrobuněčné morfogenezi. Také je ukázáno jejich možné využití v diagnostice rakoviny. Tato práce je prezentována jako soubor několika časopiseckých publikací autora této práce a jeho spolupracovníků na toto téma. Tyto články jsou doplněné jednotícími úvodními poznámkami.

Summary

This dissertation thesis deals with mechano-electrical coupling in microtubules, supramolecular structures of cellular skeleton. This mechano-electrical coupling is studied primarily in relation to vibrations if microtubule during whose the elastic deformation is accompanied with generation of oscillating electric field. Based on detailed computational model the possibility of measurement of electrical component of these vibrations is analyzed and nanotechnological approach to this issue is advocated. It is discussed that mechano-electrical vibrations of microtubules may play role in sub-cellular morhogenesis. It is also shown they may be utilized in diagnostics of cancer. The thesis is comprised of several journal papers published on this topic by the author of this thesis and his coworkers. These articles are accompanied with unifying introductory remarks.