Artificial Neural Networks Identify the Dynamic Organization of Microtubules and Tubulin Subjected to Electromagnetic Field

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Abstract: - Microtubules (MTs) are cylindrical polymers of the protein tubulin, are constituents of all eukaryotic cells cytoskeleton and are involved in key cellular functions. MTs are claimed to be involved as sub-cellular information or quantum information communication systems [1][2][3]. MTs are the closest biological equivalent to the well-known carbon nanotubes (NTs) material. We evaluated some biophysical properties of MTs by means of specific physical measures of resonance and birefringence in presence of electromagnetic field, on the assumption that when tubulin and MTs show different biophysical behaviours, this should be due to the special structural properties of MTs.

The experimental results highlighted a peculiar physical behaviour of MTs in comparison with tubulin. This paper presents the dynamic simulation of MT and tubulin when subjected to electromagnetic field. Their level of self-organization was evaluated using artificial neural networks.

Key-Words: - Microtubules, Tubulin, Nanotubes, Buckyballs, self-organization, Artificial Neural Networks, Kohonen

1 Introduction

Any tubular conductor cable, resonating mechanically, acts as a cavity antenna. The magnitude of the effect becomes significant when the frequency corresponds to the resonance frequency and in this case the output voltage can be used for receiving and transmitting radio waves. The resonance is a physical condition that occurs when a damped oscillating system is subjected to a periodic solicitation with a frequency equal to the system oscillation. A resonance phenomenon causes a significant increase in the extent of the oscillations that corresponds to a remarkable accumulation of energy within the oscillator. Recent observations and experiments on NTs have led to the development of an array of NTs able to act as antenna [4].

Due to their scale the NTs capture extremely high wavelengths. In the study of the physical properties of MTs compared with those of NTs [5] [6] we analyzed a possible reaction to microwaves, observing any ability of MTs to absorb or emit like antennas. If MTs, as well as NTs, may behave as oscillators, this could make them superreactive receivers able to amplify the signals [7]. Our experimental approach verified the existence of mechanical resonance in MTs at a frequency of 1510 MHz.

In the electromagnetic resonance experiment we identified a difference in the peak amplitude of the solution with MTs at a frequency of 1510 MHz, whereas the solution with tubulin and the control solution did not show any reaction. The lack of response in tubulin and control can be considered a hint that the peculiar structure of MTs could be the cause of the observed signal.

Moreover, we analyzed the MTs behaviour in birefringence conditions. Birefringence is an optical property of materials that arises from the interaction of light with oriented molecular and structural components. By means of a polarized light and a suitable detection apparatus, it is possible to observe the associated birefringence and, therefore, the index of orientation of MTs subjected either to transverse electric fields and to transverse and longitudinal magnetic fields. We performed in vitro experiment on different samples of MTs and tubulins, in stabilizing buffer solution, and measured the polarization under controlled conditions in order to determine different effects in the interaction of almost static electromagnetic fields. Behavioural differences observed between samples of tubulin and MTs would lead us to understand weather the cavity structure in the MT reacts in a peculiar way in response to specific stimuli or not. The analysis of the results of birefringence experiment highlights that the MTs react to electromagnetic fields in a different way than tubulin. In particular, electric field and longitudinal magnetic field show opposite effects in the two types of proteins. The achieved results, supported by statistical significance, suggest that the tubular structure of MTs might be responsible for the different behaviour in respect to free tubulins [8].
2 Dynamical simulation

In order to assess the significance of these findings, we performed a dynamic simulation of the molecular structures of tubulin and MTs subjected to different levels of electromagnetic field and in the absence of field, compared with the similar behavior in terms of carbon nanotubes (NTs) and buckyballs (BBs), globular nanostructured elements whose relationship with NTs can be compared to the relationship between tubulin and MTs. We adopted the simulation environment Ascalaph [9] due to the possibility to perform simulations for wide molecular structures with a large number of parameterizations. The simulations were carried out as follows:

- 1st simulation: zero electric field, \( A = 0 \)
- 2nd simulation: \( A = 2 \text{ V/cm}, F = 90 \text{ Hz} \)
- 3rd simulation: \( A = 90 \text{ V/cm}, F = 90 \text{ Hz} \)

The structures were immersed in water at 298.15 °K. The simulation duration was 7000 ps. We adopted the AMBER (Assisted Model Building and Energie Refinement) default force field.

The tertiary structure of tubulin was obtained from Protein Data Bank [10], MTs from the website of the NANO-D research group at INRIA Grenoble-Rhone-Alpes [11], BBs and NTs were directly obtained from Ascalaph.

After the end of simulation and a suitable dynamical optimization, the graphical visualization of the structures appears as in Fig. 1.

3 Artificial Neural Network processing

The results of the dynamical simulations were submitted to two different artificial neural networks, and their results were compared.

Artificial neural networks are intrinsically non-linear models able to classify complex patterns. In particular, the self-organizing networks as the Kohonen’s Self Organizing Map (SOM) is well-known as a natural non-linear classifiers [12][13].

The first adopted model was SONNIA. SONNIA is a powerful Artificial Neural Networks environment, very useful in the field of drug discovery and protein prediction [14]. It allows to classify a series of data sets, providing both supervised and unsupervised learning.

The output maps are represented by a set of colored boxes, one for each output neuron. The boxes configuration highlights two interesting parameters:

1. Occupancy, i.e. the number of patterns that have been mapped onto the same neuron, indicating similarities in the input domain.
2. Conflicts or conflict neurons, i.e. neurons that refer to inputs belonging to different classes.

In general, there are always at least a few conflicts such as with any other modeling technique there are false positives or false negatives.

For our case study we chose a Kohonen rectangular network structure with 9x6 neurons and a random initialization.

Besides, we used another self-organizing artificial network developed by our group, the ITSOM (Inductive Tracing Self-Organizing Map), to discriminate the dynamical behaviour of the structures under investigation, on the basis of the chaotic attractors determinated by the sequences of its winning neurons [15].

In fact an analysis on the SOM has shown that such a sequence, provided to keep the learning rates steady (instead of gradually decreasing them), constitutes chaotic attractors that repeat “nearly” exactly in time with the epochs succeeding, and that, once codified by the network, univocally characterize the input element that has determined them.

An attractor can be defined as a generalization of the steady state point, and represents the trajectory in a portion of state space where a dynamical system is attracted to [16].

We tried to highlight the presence of dynamical attractors in the described structures using MATLAB and its SIMULINK module for the dynamical systems simulation.

In the following (Fig. 2, Fig. 3) we show a comparison of the two different visualizations (SONNIA and ITSOM).

4 Results

In the conditions of zero field the tubulin shows a high occupancy value, and a rather consistent number of conflicts.

The stabilization of the neural network is achieved with the greatest difficulty with respect to all other examined structures, to highlight a lack of native dynamic organization in relationship to the other structures.

By applying a weak electric field the tubulin tends to restrict its configuration space, while maintaining similar rates of occupancy and conflict with respect to the absence of field.

With a 90 V/cm field the configuration space and the
 occupancy don’t change, but the number of conflicts is increased.

By applying a weak electric field to the BB, occupancy tends to decline, the BB tends to stabilize in a range of values. The spatial configuration tends to shrink. But as the electric field grows, the occupancy tends to return to the same levels as in the absence of field.

Although the NT structure is bigger than that of a BB, the occupancy is low and similar to the BB one, symbolizing the strong stability of the structure.

With weak electric field the situation does not change, even though there is a spatial displacement of the structure. With higher field the structure tends to go back to the positions obtained without field, although in a more distributed way, as occupancy tends to be more distributed.

Conclusions and Future Developments

The results obtained by SONNIA reflect the same behaviour observed during the Ascalaph dynamical simulation. In fact during the dynamical simulations we observed that both BBs and NTs move with a dynamic axial motion, which becomes a real pulse in the presence of electric field. The NT, which in the dynamic evolution at zero field tends to move off its initial position, with the influence of the electric field tends to return to the starting position and to stabilize.
The behavior of the neural network reflects this trend, which shows the extreme regularity of these nanostructures and an interesting already known behavior of NT in the presence of electric field.

The tubulin, despite its symmetric structure, seems to have different internal forces that tend to resist a dynamic stabilization. However, in the presence of electric field, although it tends to squash, it does not show any particular reaction.

The dynamic simulation confirms the lack of specific characterization. The figures emerged from the neural simulation show a MTs dynamic organization much stronger than the tubulin one, that is not altered by the presence of electric field even in its spatial configuration.

It is worth noting, however, a very significant reduction of conflicts, which would indicate a dramatic increase in the spatial organization. On the other hand, the graphs obtained by the ITSONM network confirm the SONNIA neural network analysis and the dynamic simulations. The attractors generated by BB both in the absence of field and with low electric field are extremely cyclical and regular, even though with higher field it tends to present a regular compactness, and to broaden its values, as described by the SONNIA output.

The attractor regularity is clearly present even in the NTs, and the electric field tends to increase both spatial range and regularity. The tubulin, which is initially well-structured (although with a much more complex pattern of NT and BB), maintains a structured shape even in presence of electric field, although with an increase of disorder. The MTs, however, despite their structural complexity, show a strong dynamic stability, which the electric field, after an initial transient, improves significantly. The field increase further stabilizes the structural dynamics and the spatial configuration of MTs.

Ultimately all three methods converge in emphasizing the dynamic stability of these four structures, but show that only NTs and MTs exhibit a significant behavior in presence of electric field, in the direction of a stronger structural and spatial organization.

These results confirm those already obtained in the cited previous experiments on real samples of tubulin and MTs in conditions of resonance and birefringence.

For this reason, the research on these interesting structures will continue with further studies.

An experiment to search for superradiant behavior in MTs is underway in collaboration with the Department of Materials Science, University of Milano-Bicocca. However, the use of simulation methods can help to motivate at a microscopic level the experimental evidences and justify the agreement with theoretical assumptions.

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References:
[10] Protein Data Bank: http://www.rcsb.org/pdb/home/home.do