THE ANNALS OF "DUNĂREA DE JOS" UNIVERSITY OF GALAȚI
FASCICLE V, TECHNOLOGIES IN MACHINE BUILDING,
ISSN 1221- 4566, 2011

HARMFUL EFFECT OF CHEMICAL COMPOUNDS USED IN AGRICULTURE ON THE HUMAN HEALTH

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ABSTRACT

Recently, it was observed an increased usage of microtubules-depolymerizing agents, such as pesticides and anti-fungal or antiparasitic compounds to help the production of the agriculture products. All these chemical compounds are used in agriculture and have a destroying effect on human health, leading on the appearance of neurodegenerative diseases. For this reason, many researches are focused on the understanding in detail the correlation between the general mechanism of action for pesticides and microtubules deploymerization process which is present in the human cell. In this paper, we present the analysis of the mechanism involved in depolymerizing of the microtubules structure through molecular dynamic simulations. This mechanism is time dependent and contributes to the deterioration of the neuronal transport carried-out by the kinesin and microtubules, leading to a miss carriage of the Ca^{+} ions to the nerve synapse. Aided by molecular dynamics simulations, the health monitoring of the microtubules is carried out by measuring the evolution of some mechanical parameters which characterize the integrity of the microtubules and their capacity to be transporters for the neuronal signs.

KEYWORDS: pesticides, molecular dynamic simulations, health monitoring of the microtubules

1. Introduction

Recently, it was observed an increased usage of microtubules-depolymerizing agents, such as pesticides and anti-fungal or antiparasitic compounds to help the production of the agriculture products [3, 9]. All these chemical compounds are used in agriculture and have a destroying effect on human health, leading on the appearance of neurodegenerative diseases. One of the most used chemical compounds in agriculture with destroying effect on human health is rotenone. This compound is a natural product extracted from bean found in subtropical and tropical regions from South America and South-East Asia, appertaining to iso-flavons class.

Microtubules (MTs) degradation by rotenone has a harmful effect on dopaminergic neurons due to appearance of a constant loosing of neurotransmitters from vesicles (affection of vesicular transport) and increasing of oxidation dopamine process (neurons death). All these causes which contribute to microtubules degradation have an important role in appearance of Parkison's disease (PD) [1, 2, 3, 4, 5, 6, 7, 8]. Tubulin folding process depends on ATP hydrolysis and presence of rotenone determines the reduction of ATP production, which has a devastator effect on tubulin folding reaction. In this way tubulin structure is deteriorate and it appears the depolymerization process of microtubules [3]. For this reason, many researches are focused on the understanding in detail the correlation between the general mechanism of action for pesticides and

microtubules deploymerization process which is present in the human cell. In this paper, we present the analysis of the mechanism involved in depolymerizing of the microtubules structure through molecular dynamic simulations. This mechanism is time dependent and contributes to the deterioration of the neuronal transport carried-out by the kinesin and microtubules, leading to a miss carriage of the Ca^{+} ions to the nerve synapse. Aided by molecular dynamics simulations, the health monitoring of the microtubules is carried out by measuring the evolution of some mechanical parameters which characterize the integrity of the microtubules and their capacity to be transporters for the neuronal signs.

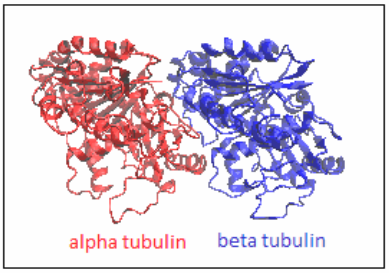
2. Effect of rotenone on the microtubules integrity

When rotenone formulations intended for pesticides applications are administered to rivers and streams for the highly selective removal of invasive fishes, the resulting concentrations of ingredients, including rotenone, are of little concern regarding human health, or the welfare of other mammals and birds that may come in contact with the rotenone-treated water. Rotenone is fairly quickly detoxified by degradation pathways involving photolysis and hydrolysis, and has a short half life in the environment. Excess rotenone may be converted to products of lower toxicity by introduction of potassium permanganate. The connection between oral ingestion of rotenone and Parkinson's disease is extremely tenuous, mainly because this route of ingestion effectively detoxifies rotenone by enzymatic, bacterial, and hydrolytic reactions in the gut that lead to rapid excretion of degradation byproducts. Other solvent components of rotenone formulations when diluted to the low concentrations intended for pesticidal applications, are substantially below the safe concentrations set for drinking water contaminants by the EPA.

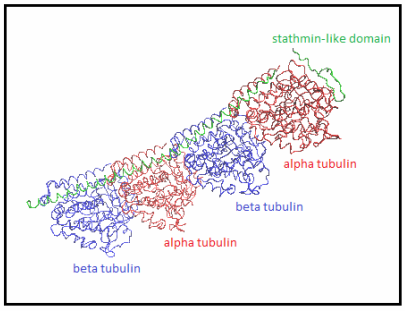
In vitro, rotenone has been shown to produce cell apoptosis, accumulation and aggregation of synuclein and ubiquitin, oxidative damage, and endoplasmic reticulum stress. In vivo, chronic systemic exposure to rotenone has been used to model PD in the rat [10, 11], which it was shown to induce dopaminergic neurodegeneration, Parkinson like behavior, and the occurrence of cytoplasmic inclusions similar to the Lewy bodies [1]. However, several observations have questioned the reliability of this model. Significant differences were observed between rats of the same strain, making it difficult to test new pharmacological treatments. It has been reported recently that rotenone leads to nonselective neuronal death in this model [12], whereas another study suggested that this may in part depend on the mode of administration of the pesticide [13]. Finally, it is unknown whether the movement phenotypes of the rotenone treated rat result from dopamine deficiency and thus can be used to assess the degree of dopaminergic degeneration.

3. Algorithm for investigation the effect of rotenone on the microtubules

The goal of this article is to develop an algorithm to measure mechanical properties of proteins affected by harmful effect of pesticides. It is used numerical simulation to atomistic level (MD simulations) on proteic chains obtained from Protein Data Bank (PDB). Proteins structures are represented by residues coordinates and distances between them. To evidence the interaction between residues, there are used CHARMM field parameters. In this paper, molecular dynamics simulations were performed for normal microtubules structure and tubulin-colchicine: stathmin-like domain complex to evaluate stiffness of these two proteins (Fig. 1. (a), (b)). Alpha-beta tubulin dimer is composed from 874 amino acids (440 α-tubulin amino-acids and 427 β-tubulin amino-acids) and presents 7406 C-C bonds. The second analyzed structure, tubulin-colchicine: stathmin-like domain complex, contains a total residues number equal to 1934 amino acids (902 α-tubulin amino acids, 890 β-tubulin amino-acids and 142 stathmin-4 amino acids) and respectively, 14394 C-C bonds.



(a)



(b)

Fig. 1. *Structures used in NAMD simulations represented in VMD editor. (a) Normal microtubules structure. (b) Tubulin-colchicine: stathmin-like domain complex*

3.1. Method and materials

To evidence the mechanism involved in depolymerizing of the microtubules structure, we studied some mechanical parameters, comparing the normal microtubules structure (PDB ID: 1TUB) with affected one by rotenone. The normal microtubules structure has an alpha tubulin and beta one, which is an atomic model of the alpha-beta tubulin dimer fitted to a 3.7 Å density map obtained by electron crystallography of zinc-induced tubulin sheets [14]. Due to similar toxic action of both rotenone and colchicine, it was studied tubulin-colchicine: stathmin-like domain complex (PDB ID: 1SA0). This structure was obtained with a 3.5 Å resolution and contains tubulin in complex with colchicine and with the stathmin-like domain (SLD) of RB3. In this molecule appears the interaction of RB3-SLD with two tubulin heterodimers in a curved complex capped by the SLD amino-terminal domain, which prevents the incorporation of the complexed tubulin into microtubules [15]. A comparison between normal tubulin structure and tubulin-colchicine: stathmin-like domain complex, it shows some changes of tubulin subunits, which pass from a straight conformation to a curved one. These changes are correlated to the loss of lateral contacts and thus, it can be explained how depolymerization process appears suddenly. Algorithm used to calculate the values of microtubules stiffness is presented in Fig. 2.

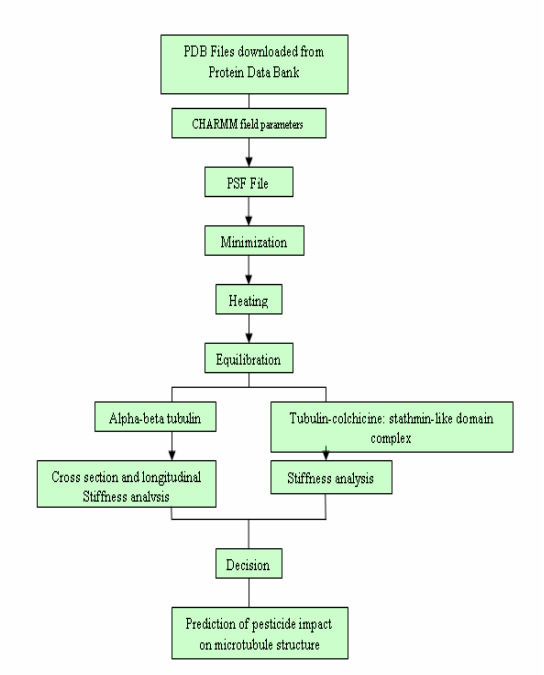


Fig. 2. *Proposed algorithm for prediction the harmful impact of pesticides on the MTs health through out monitoring the cross-section and longitudinal stiffness as a measure of MTs disintegration*

One of the most important mechanical parameters which can give information about causes of microtubules depolymerization is microtubules stiffness. To evaluate stiffness of structures used in our NAMD simulation, we performed equilibration process during 100 ps for each structure.

In order to evaluate the normal microtubules structure stiffness using NAMD simulation, we have prepared our system for minimization (2 ps), heating (300 ps) and equilibration (100 ps) with periodic boundary conditions. This structure of dimensions (101.176 x 58.102 x 60.976 $Å^3$) was placed into a water box of dimensions (110.169 x 68.095 x 70.971 $Å^3$) and the water layer of 5 Å, in which it was used TIP3P model for water molecules and there were added Na^+ and Cl^- counter ions at a concentration of 30 mM (Fig. 3).

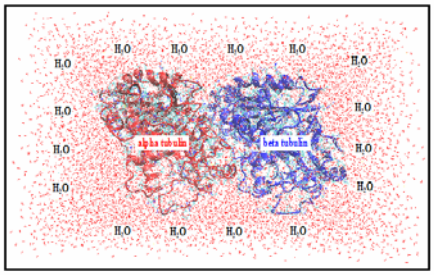


Fig. 3. *Normal microtubules structure placed in a water box represented in VMD editor*

The total system contains 48261 atoms, 12959 normal microtubules atoms, 35302 water atoms, 49 Na^+ ions and 17 Cl^- ions. The van der Waals interactions were calculated with a cutoff of 12 Å and the electrostatic interactions were calculated using the Particle Mesh Ewald method [16]. A multiple time-stepping algorithm [17] was used a 2 fs step. NAMD simulations were made in an NPT ensemble (pressure = 1atm, temperature 300 K), in which the pressure was controlled by the hybrid Nosé - Hoover Langevin piston method [18, 19] and the temperature was controlled using Langevin dynamics.

The same procedure was used to analyze the tubulin-colchicine: stathmin-like domain complex stiffness. A structure of dimensions (178.85 x 159.4 x 79.31 $Å^3$) was placed into a water box of dimensions (188.84 x 159.4 x 89.31 $Å^3$) and the water layer of 5 Å, in which it was used TIP3P model for water molecules and there were added Na^+ and Cl^- counter ions at a concentration of 30 mM. The analyzed system contains 212416 atoms, 63292 tubulin-colchicine: stathmin-like domain complex atoms, 149124 water atoms, 70 Na^+ ions and 31 Cl^- ions.

3.2. Evaluation of microtubules health

Evaluation of microtubules health could be done through measuring their stiffness as a measure of the

integrity of MTs. When a pesticide attacks MTs, their chemical bonds are affected so that the stiffness suffers modifications. To determine the stiffness, a new method was deployed based on Matsushita et al. method used to calculate the actin filaments stiffness [20]. It is supposed that the deformation of the MTs is given by different modes among which the most important is deformation along the MTs axis. The energy caused by this deformation is assumed to be equal with the Boltzmann energy, as written in the following equation:

$$\frac{1}{2}k_{ext}^{\Delta t}(t)\left\langle (L(t)-\langle L(t)_{\Delta t}\rangle)^2\right\rangle_{\Delta t}=\frac{1}{2}k_B T \quad (1)$$

where, k_B = the Boltzmann constant equal to 1.3806488(13)*10^{-23} J/K; T = the absolute temperature of thermodynamic system; $<>_{\Delta t}$ = average over time of fluctuating under force zero; Δt = 10, 20, 40, 60, 80, 100 ps; $k_{ext}^{\Delta t}(t)$ = the extensional spring constant of microtubules.

The length of alpha-beta tubulin dimer, $L(t)$, is defined as,

$$L(t)=x_{plus}(t)-x_{minus}(t) \quad (2)$$

where, $x_{plus}(t)$ is the position in the x-axis of the centre mass of beta chain at the plus end, and $x_{minus}(t)$ is the one of alpha chain at the minus end.

Meanwhile, the length of tubulin-colchicine: stathmin-like domain complex, $L(t)$, is defined as,

$$\begin{aligned} L(t)&=\sqrt{a^2+b^2+c^2}\\ a&=x_{plus}(t)-x_{minus}(t)\\ b&=y_{plus}(t)-y_{minus}(t)\\ c&=z_{plus}(t)-z_{minus}(t) \end{aligned} \quad (3)$$

where, $x_{plus}(t)$ is the position in the x-axis of the centre mass of alpha chain at the plus end, and $x_{minus}(t)$ is the one of beta chain at the minus end; $y_{plus}(t)$ is the position in the y-axis of the centre mass of alpha chain at the plus end, and $y_{minus}(t)$ is the one of beta chain at the minus end; $z_{plus}(t)$ is the position in the z-axis of the centre mass of alpha chain at the plus end, and $z_{minus}(t)$ is the one of beta chain at the minus end.

In this paper, the microtubules stiffness was evaluated based on MD simulation (performed around 100ps), using the extensional spring constant of microtubules from equation (1) and conventional units. Extensional microtubules stiffness was calculated as the value per 1 μm length. Thus, the 1 μm-long apparent extensional stiffness, $K_{ext}^{\Delta t}$, per unit length of microtubules is given by,

$$K_{ext}^{\Delta t}=\frac{\langle L(t)\rangle_{\Delta t}}{1\mu m}k_{ext}^{\Delta t} \quad (4)$$

4. Results and discussion

Using NAMD molecular dynamic software released by University of Illinois, USA [21], MTs stiffness in two cases (alpha-beta tubulin dimer structure and tubulin-colchicine: stathmin-like domain complex) were calculated. MD simulations are composed of two steps, minimization and equilibration. Minimization step consists of searching the energy landscape of the molecule for a local minimum, such as place in which the molecule is relaxed, by systematically varying the positions of atoms and calculating the energy. Equilibration involves molecular dynamics, whereby Newton Second Law is solved for each atom in the system to dictate its trajectory.

To analyze the extent to which our system has equilibrated, we plotted root mean square deviation (RMSD) versus time (Fig. 4. (a), (b)).

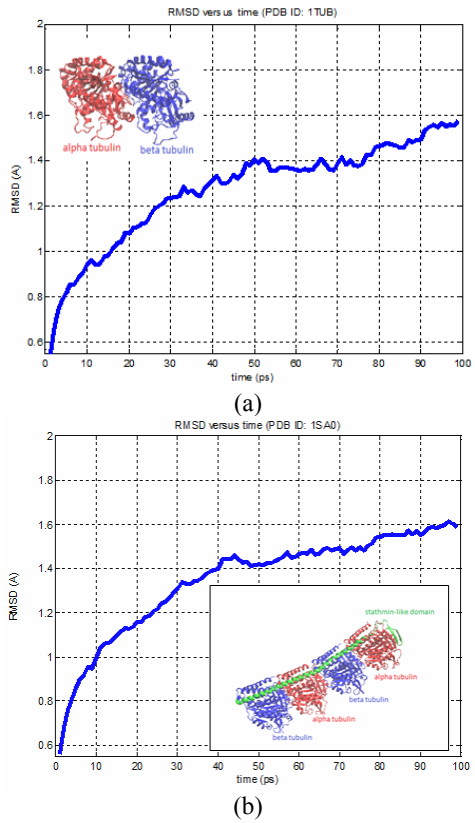


Fig. 4. *RMSD versus time for equilibrated microtubules structures used in our NAMD simulations. (a) Alpha-beta tubulin dimer structure - saturation value for RMSD is achieved around 60 ps. (b) Tubulin-colchicine: stathmin-like domain complex - saturation value for RMSD is achieved around 70 ps*

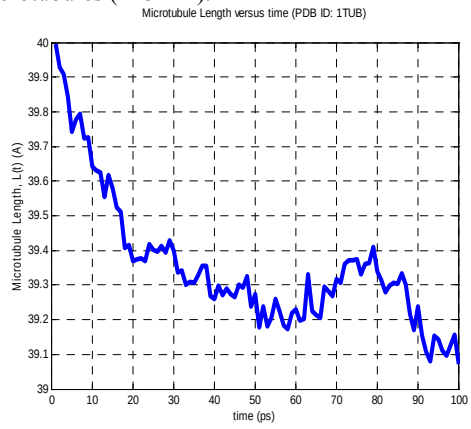
The RMSD offers information about the amount by which a given selection of our molecule deviates from a defined position in space.

By plotting RMSD the equilibrium of the MTs in water is calculated meaning that it is achieved a geometrical configuration characterized by a minimum energy. Saturation of RMSD it shows a steady-state of MTs where a further step of MD could be applied. In the MTs analyzed structures (alpha-beta tubulin dimer structure and tubulin-colchicine: stathmin-like domain complex), the plot of RMSD is presented and the equilibrium was achieved after 100 ps.

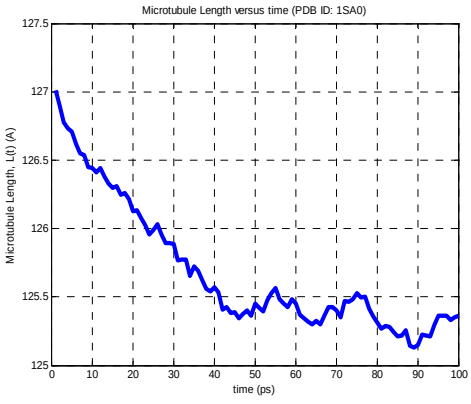
Two essential mechanical parameters were evaluated in this study: extensional stiffness and respectively, Young's modulus of microtubules, E, using a roughly estimation given by equation (5).

$$E = \frac{k_{ext} L}{A} \tag{5}$$

where, k_{ext} = the extensional spring constant of microtubules; L = the average microtubules length; A = the equivalent cross-sectional area of microtubules (~25nm^2).



(a)



(b)

Fig. 5. *Evolution of the microtubules structure length (a) health MTs and (b) affected MTs*

After 100 ps MD simulations, alpha-tubulin dimer has an average length equal to 39.3547 Å, an extensional stiffness around 0.0290 N/m ± 0.0164 and a Young's modulus around 1.79 x 10^9 N/m^2, which is close to the value obtained by Sept and MacKintosh, 2010 (for tubulin E= 2.2 x 10^9 N/m^2) [22]. The evolution of microtubules structure length in time is showed in Fig. 5. (a),(b).

Based on the evolution length obtained following MD simulation, stiffness for the two cases was calculated using equation (5). The obtained values for the stiffness are 0.0290 N/m ± 0.0164, E = 1.79 x 10^9 N/m^2 (health MTs) and respectively, 0.0147 N/m ± 0.0085, E = 0.958 x 10^9 N/m^2 (affected MTs).

In comparison with alpha-beta tubulin dimer, the structure affected by rotenone presents a lower stiffness due to beginning of depolymerization of microtubules by loosing of lateral contacts from this structure. This conclusion allows monitoring of the MTs health and the disintegration stage caused by presence of pesticides.

5. Conclusions

Microtubules present a significant role in intracellular transport carrying various organelle inside of cells and depolymerizing of these structures by action of different chemical compounds has a destroying effect on human health. Aided by molecular dynamics simulations, we measured the evolution of some mechanical parameters which characterize the integrity of the microtubules and their capacity to be transporters for the neuronal signs. Comparison made between the stiffness value of beta-alpha tubulin dimer and respectively of tubulin-colchicine: stathmin-like domain complex, showed how can be influenced the health monitoring of the microtubules. In the present paper, a molecular dynamic analysis of the MTs health was carried out. An indirect method was used to estimate the impact of pesticides on the MTs. The measure of stiffness as an indicator of dezintegration of MTs was done.

ACKNOWLEDGEMENTS

The work of Simona Patriche was supported by Project SOP HRD - EFICIENT 61445 and ID1758/2008.

REFERENCES

[1] Betarbet, R., Sheer, T.B., Mackenzie, G., Garcia-Osuna, M., Panov, A.V., Greenamyre, T., *Chronic systemic pesticide exposure reproduces features of Parkison's disease*, Nature Neuroscience, 3(12), 2000, pag. 1301-1306.

[2] Giasson, B.I., Lee, V.M.Y., *A new link between pesticides and Parkison's disease*, Nature Neuroscience, 3(12), 2000, pag. 1227-1228.

[3] Feng, J., *Microtubule: A common Target for Parkin and Parkison's Disease Toxins*, The Neuroscientist, 12(6), 2006, pag. 469-476.
[4] Jiang, Q., Yan, Z., Feng, J., *Neurotrophic Factors Stabilize Microtubules and Protect against Rotenone Toxicity on Dopinaminergic Neurons,* J. of Biological Chemistry, 81 (39), 2006, pag. 29391-29400.
[5] Ren, Y., Feng, J., *Rotenone selectively kills serotoenergic neurons through a microtubule-dependent mechanism*, J. of Neurochemistry, 103, 2007, pag. 303-311.
[6] Borland, M.K., Trimmer, P.A., Rubinstein, J.D., Keeney, B., Mohanakumar, K.P., Liu, L., Bennet, P.J., *Chronic, low-dose rotenone reproduces Lewy neuritis found in early stages of Parkison's disease, reduces mitochondrial movement and slowly kills differentiated SH-SY5Y neural cells*, Molecular Neurodegeneration, 2008, pag. 1-12.
[7] Drolet, R.E., Cannon, J.R., Monterro, L., Greenamyre, J.T., *Chronic rotenone exposure reproduces Parkison's disease gastrointestinal neuropathology*, Neurobiology of Disease, 36, 2009, pag. 96-102.
[8] Choi, W.S., Palmiter, R.D., Xia, Z., *Loss of mitochondrial complex I activity potentiates dopamine neuron death induced by microtubule dysfunction in a Parkison disease model*, JCB, 192(5), 2011, pag. 873-882.
[9] Saybasili, H., Akkentli, F., *Rotenone is a pesticide controlling the habitat quality of aquatic ecosystems and has a negative impact on neuron activity*, Review of Hydrobiology, 4, 2011, pag. 1-16.
[10] Ryu, E.J., Harding, H.P., Angelastro, J.M., Vitolo, O.V., Ron, D., Greene, L.A., *Endoplasmatic reticulum stress and the unfolded protein response in cellular models of Parkison's disease*, J. of Neuroscience, 22, 2002, pag. 10690-10698.
[11] Sheer, T.B., Betarbet, R., Greenamyre, J.T., *Environment, mitochondria, and Parkison's disease*, The Neuroscientist, 8(3), 2002, pag. 192-197.
[12] Hoglinger, G.U., Feger, J., Prigent, A., Michel, P.P., Parain, K., Champy, P., Ruberg, M., Oertel, W.H., Hirsch, E.C., *Chronic systemic complex I inhibition induces a hypokinetic multisystem degeneration in rats*, J. of Neurochemistry, 84, 2003, pag. 491-502.
[13] Sheer, T.B., Betarbet, R., Testa, C.M., Byoung, B.S., Richardson, J.R., Kim, J.H., Miller, G.W., Yagi, T, Greenamyre, J.T., *Mechanism of toxicity in rotenone models of Parkison's disease*, J. of Neuroscience, 23, 2003, pag. 10756-10764.
[14] Nogales, E., Wolf, S.G., Downing, K.H., *Structure of the alpha beta tubulin dimer by electron crystallography*, Nature, 391, 1998, apg. 199-203.
[15] Ravelli, R.B., Gigant, B., Curmi, P.A., Jourdain, I., Lachkar, S., Sobel, A., Knossow, M., *Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain*, Nature, 428, 2004, pag. 198-202.
[16] Darden, T., York, D., Pedersen, L., *Particle mesh ewald – an N.log (N) method for Ewald sums in large systems*, J. of Chemical Physics, 98, 1993, pag. 10089-10092.
[17] Sclock, T., Skeel, R.D., Brunger, A.T., Kalek, L.V., Board, J.A., Hermanes, J., Schulten, K., *Algorithmic challenges in computational molecular biophysics*, J. of Computational Physics, 151, 1999, pag. 9-48.
[18] Hoover, W.G., *Canonical dynamics-equilibrium phase-space distributions*, Physical Review A, 31, 1999, pag. 9-48.
[19] Martyna, G.J., Klein, M.L., Tuckerman, M., *Nosé-Hoover chains – the canonical ensemble via continuous dynamics*, J. of Chemical Physics, 97, 1992, pag. 2635-2643.
[20] Matshushita, S., Adachi, T., Yasuhiro, I., Masaki, H., *Evaluation and torsional stiffness of single actin filaments by molecular dynamics analysis*, J. of Biomechanics, 43 (16), 2010, pag. 3162-7.
[21] Phillips, J.C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R.D., Kale, L., Schulten, K., *Scalable molecular dynamics with NAMD*, J. of Computational Chemistry, 26, 2005, pag. 1781-1802.
[22] Sept, D., MacKintosh, F.C., *Microtubule Elasticity: Connecting All-Atom Simulations with Continuum Mechanics*, Physical Review Letters, 104, 2010, pag. 018101-1-4.

Efectul nociv al compuşilor chimici folosiți în agricultură asupra sănătății umane

—Rezumat—

În ultima vreme, s-a observat o însemnată utilizare a agentilor de depolimerizare a microtubulilor, cum ar fi pesticidele si compusii antifugici sau cei antiparazitanti pentru a ajuta productia produselor agricole. Toti acesti compusi chimici sunt utilizati în agricultură si au un effect devastator asupra sănătătii umane, conducând la aparitia bolilor neurodegenerative. De aceea, multe cercetări sunt axate pe întelegerea în detaliu a corelatiei dintre mecanismul general de actiune a pesticidelor si procesul de depolimerizare a microtubulilor, ce este prezent în celula umană. În această lucrare, prezentăm analiza mecanismului implicat în depolimerizarea structurii microtubulilor folosind simulările de dinamică moleculară. Acest mechanism este dependent de timp si contribuie la deteriorarea transportului neuronal realizat de către kinezină si microtubuli, conducând la insuccesul transportului ionilor de Ca^{+} la sinapsele nervoase. Cu ajutorul simulărilor de dinamică moleculară, monitorizarea stării de sănătate a microtubulilor este realizată prin măsurarea evolutiei câtorva parametri mecanici, ce caracterizează integritatea microtubulilor si capacitatea lor de a fi transportatori pentru semnalele neuronale.