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Computer-aided molecular design of nanocontainers for inclusion and targeted delivery of bioactive compounds

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Nanotubes may serve as non-immunogenic containers of biocompounds and vehicles for selective delivery to the cell membrane. These two properties – selective inclusion and transportation – are interconnected and establish the main challenge in nanovehicle design. Molecular dynamics (MD) simulation of systems containing a carbon nanotube, water, a lipid bilayer, and a molecule to be delivered is a way to explore the problem. Absorptive properties of the nanotube were studied using the example of a pentadecapeptide and cholesterol. The uptake of these molecules turned out to be susceptible to details of the nanotube's interfacial structure and functional groups of the molecules contacting the nanotube. Incorporation of the peptide was found to be a spontaneous process and considered as a model self-assembly of a nanodevice, further referred to as a nanosyringe. For the design of the nanosyringe the steered molecular dynamics (SMD) approach was used. Release of the peptide was performed by expulsing it through the lipid membrane. The conformational state of the peptide was studied in view of chemical stability of the substance under shock action. In principle, styling the nanotube (by adding functional groups or ligands) may achieve the selectivity of the nanotube's landing area on the cellular membrane and implement it in drug delivery system construction.

Key words: Molecular dynamics – Nanotubes – Biomembranes – Peptides – Drug delivery.

Study of interaction of nanotubes with biological molecules is of great practical importance in connection with the problem of selective delivery of drugs to the cells. Some successful specimens of nanotubes technology include a recent light-actuated nanovalve [1], targeted delivery of amphotericin B to cells by using functionalized carbon nanotubes [2]. Model investigations in this area have already begun as well. Thus, a simulation of penetration of a one-strand DNA olygonuclotide into a carbon nanotube in aqueous medium is reported in [3]. The authors of [4] studied the passage of an RNA under the action of an applied force through the holes in short nanotubes constituting a monolayer. The adsorption of amylose on a nanotube in water and the penetration of the first into the nanotube were studied in [5]. One of the most fascinating examples of molecular simulations is probably a rather simplified coarse-grain modeling of a nanosyringe prototype, which is, in fact, a nanotube capable of penetrating a membrane [6]. Molecular dynamics (MD) and molecular modeling play an important role in hypothesis generation, formulation of basic principles and the trend of development of new materials. For instance, four ranges which have the greatest potential of industrial effect in future years are recommended for modeling in [7]: new modeling strategies for complex material systems, simulation-based design of new functional materials, computer-guided methods for nanotechnology, and modeling of biological and biomimetic materials. MD methods are widely applied to investigation of fundamental problems of natural sciences, and also in applied problems of molecular bioengineering, biotechnology, nanotechnology, material science, etc. [8, 9]. Drug design is even more an open field for MD simulation. Solution of a system with a large number of classical equations of motion for atomic particles is carried out, as a rule, with the use of the Verlet difference scheme. The force field is assigned by a system of atom-atomic potentials, which are specially gauged for a particular type of molecular objects (biopolymers, minerals, alloys, etc.). Usually, special algorithms for maintaining a constant temperature and pressure (or volume) are also used.

In this paper, the MD approach employing an all-atom force field and special procedures was used to model and design nanocontainers for inclusion of biocompounds and delivery through lipid membrane. A carbon nanotube thus revealed a potential for inclusion of rather small molecules such as polypeptides and cholesterol. A nanosyringe (*Figure 1*) based on a capped nanotube with a nanoagent capable of ejecting an active molecule out of the nanotube [10] was modeled in various media including a lipid membrane. A set of swelling model spheres emulated the releasing compound (it is supposed that some external signal such as light is needed to activate release). For this purpose a modified variant of steered molecular dynamics (SMD) [11, 12] was developed. Moreover, spontaneous self-assembly of the nanosyringe was studied. To maintain constant temperature conditions, we used a collisional thermostat [13, 14], which does not lead to nonlinear attractor regimes distorting statistical equilibrium in energy distribution by degrees of freedom [15, 16].

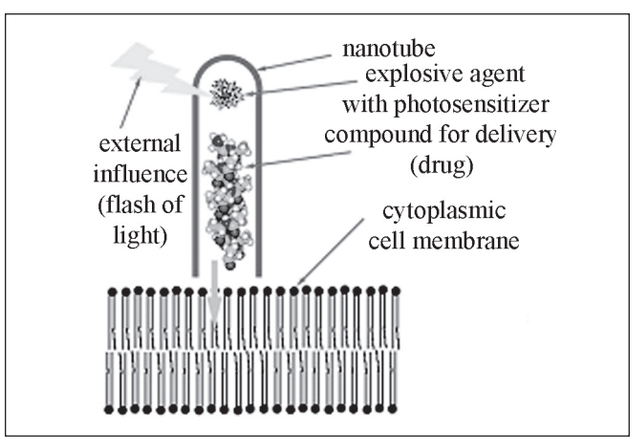

Figure 1 - Schematic representation of the nanosyringe in action.

I. MATERIALS AND METHODS

MD simulations were carried out in the PUMA program [13, 14], which was specially modified in order to include steering forces [17]. The system of classical equations of atom motion was solved in the Amber 1999 force field [18]. Periodic boundary conditions were imposed on the system. A virtual collision medium was used to maintain a constant temperature of 300 K [13]. The mean frequency of collisions of virtual particles was 10 ps^{-1}, and the mass of virtual particles was 1 amu. The systems without water and the systems simulated at a temperature higher than 300 K were simulated in NVT-ensemble (constant volume). In the aqueous systems (with the only exception for temperatures above 300 K), the constant-pressure condition was imposed. It was achieved by using the Berendsen barostat [19]. The mean frequency of barostating was 1 ps^{-1}. Except for the systems containing lipids, the barostating was isotropic and the mean value of pressure was kept constant at 1 atm. Fluctuations of volume, pressure and temperature were used to verify that the system had reached a local equilibrium. The cutoff for Van der Waals interactions was 16 Å, for Coulomb ones - 20 Å. The smoothing procedure for Van der Waals interactions involved multiplication of the Lennard-Jones potentials by the smoothing (switching) function W(r):

$$W(r)=\begin{cases}1, r \le R_{on}\\ \dfrac{\left(R_{off}^2-r^2\right)^2\left(R_{off}^2-3R_{on}^2+2r^2\right)}{\left(R_{off}^2-R_{on}^2\right)^3}, R_{on}<r<R_{off}\\ 0, r \ge R_{off}\end{cases}$$

where r is the distance between the interacting atoms, R_{on} parameter was 15 Å, and R_{off} was 16 Å. The Coulomb potential was scaled by the screening function:

$$W(r)=\begin{cases}\left(1-r/R_{off}\right)^2, r \le R_{off}\\ 0, \qquad r > R_{off}\end{cases}$$

The dielectric constant was assumed to be unity. The step of numeric integration was 1 fs.

1. Nanotube

A 580-carbon (10,10) nanotube 34.4 Å long with a diameter of 13.7 Å was formed out of 14 graphite rims, each of 10 carbon hexagons, spliced together one atop of another. In the case of the nanosyringe construction, 50 carbon atoms were further added to form a nanotube capped at one side. Likewise, 140 atoms were evenly deleted for making the initial nanotube shorter in simulations with cholesterol. The carbon atoms are uncharged and the parameters of interatomic interactions are totally those of CA (aromatic) atoms in the force field.

2. Internal components of the nanotube

With the exception of surrounding water molecules, there were only two internal components as such: pentadeca-alanine (effective diameter 8.5 Å and length of alpha-helix 25 Å) and cholesterol (effective diameter in gaunt conformation ca. 7 Å, length varies from 10 to 27 Å). The carbon atoms of the latter are all of CT type (sp^3 hybridization) except two, which are sp^2 carbons. One of those two that has a hydrogen atom (HA type) was assigned CM type, the other was considered as CB carbon. All the other atoms have corresponding types in the Amber 1999 force field, which was built specially for amino acids and nucleotides. The partial charges were calculated by the unrestricted Hartree-Fock method in a 6-311G basis set with one d-type and one f-type heavy atom polarization functions. The charges calculated according to Mulliken were taken into account and equalized in the groups of symmetrical atoms. In these quantum-chemical calculations, the GAMESS [20] package was used. Since the pentadeca-alanine was constructed out of fifteen equal alanine residues, it had, in fact, one hydrogen atom absent at the amino group of the first-in-chain residue and one hydroxyl group missing at the opposite end of the chain.

3. Van der Waals spheres

Beside the molecules mentioned in the previous subsection, a set of eight Van der Waals spheres with a mass of 99 amu, interacting with all atoms only by means of Van der Waals forces, was applied in nanosyringe-related simulations. Their radius being varied, it has the minimum value of 2 Å. The energetic constant _ of their interactions with atoms (as taken for a pair consisting of two Van der Waals spheres, which is of the same type) is 0.15 kcal/mol. Swelling at a constant rate, the spheres imitated a nanoexpulsing agent inside the nanotube. The rate of their radii augmentation was either 0.25 or 0.5 Å/ps. The period of time when the spheres were swelling was 26 or 13 ps, respectively. After the swelling the radii were reduced to the initial value at once.

4. Lipid bilayer

The membrane bilayer was composed of 1-pamitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) and contained 64 lipid molecules. In the initial structure, the direction of the longest molecular dimension was perpendicular to the membrane plane. The initial surface density of lipids was 66 $Å^2$, which was close to the experimental value (62-68 $Å^2$ [21-24]).

The parameters of potentials for the double bond in oleic residue and partial charges in POPC were taken from [25-28]. The TIP3P [29] water model was used; the valence bonds and valence angles in water molecules were not fixed, but were instead determined by the corresponding potentials. In the initial configuration, water molecules were placed at a distance not less than 2.3 Å from the outermost atoms of the membrane. The degree of solvation was 44 water molecules per one lipid molecule (full hydration of POPC requires at least 27 water molecules per lipid [30]). Later on, the total number of water molecules was diminished due to the insertion of the nanotube so that the total system's volume was kept constant.

The POPC bilayer was equilibrated for 1 ns under NP_zAT-conditions (constant pressure in the normal direction to the bilayer, constant area of the simulation box and temperature). Mean lateral pressure component was -330 atm. The mean membrane normal component was -118 atm. This is caused by effects of surface tension. The latter is susceptible to parameters of the force field which are not yet known well enough. In order to compensate the effects of surface tension it is necessary to use negative pressure while barostating, which was discussed many a time earlier [31-34]. According to the estimate [32], surface tension of 56 dyn/cm in a bilayer of dipalmitoylphosphatidylcholine results in negative lateral pressure of ca. -100 atm [35] in compliance with the following formula:

$$\gamma = \int_{z_1}^{z_2}\left[P_N(Z)-P_T(Z)\right]dz$$

where γ is the surface tension, Z axis the normal to the membrane surface, P_N the pressure in normal direction, P_T the pressure which is tangential to the membrane surface, and z_1 and z_2 the membrane boundaries (including the first layer of hydration) along Z axis. The existing difference between the pressure value used in the paper and the theoretical estimate may be related to inaccuracy in force field parameters. At the operative part of the trajectory, barostating at a mean pressure of $P_x = P_y = -260$ atm in the XY plane (which is parallel to the membrane plane) and $P_z = 1$ atm in the normal direction was performed.

After the equilibration, basic membrane characteristics such as surface tension, surface density of lipids, radial distribution functions of atoms in the bilayer plane, thickness of the bilayer, distribution of atomic groups along the membrane normal, and parameters of order for the lipid chains agree in general with the data obtained in other computer investigations and experimental data [21-24, 31, 36-43].

5. Inclusion simulations

The cholesterol molecule oriented with its hydroxyl end towards the uncapped nanotube was put at a distance of 4 Å above an opening of the latter. In one case, the system was hydrated with 5,620 molecules of TIP3P water, including the nanotube's cavity. In the other case, water was removed from the cavity and the total amount of the water became 84 molecules less. The initial size of the simulation cell was 56.1 × 56.1 × 56.1 Å (the total number of atoms was either 17,374 or 17,122).

The capped nanotube and the peptide were placed in vacuum 30 Å away from each other. The approach was observed at three different temperatures: 300, 1,000 and 2,000 K. Such high temperatures as 1,000 and 2,000 K were used to speed up over-barrier transitions, considering that the valence bonds do not break at all in MD procedures. The conformation of the peptide in connection with its orientation in the nanotube in the incorporated state was also studied.

6. Nanogun simulations

The system is comprised of the nanotube with the polypeptide and Van der Waals spheres inside. The contents of the nanotube were expelled at 300 K to three media: vacuum, water and lipid membrane. The number of water molecules in the last two simulations was 2,594. The nanotube adjoined the lipid bilayer at right angle. The conformation of the peptide during the nanoshot was traced.

II. RESULTS AND DISCUSSION

1. Incorporation of cholesterol

All the simulations of the cholesterol incorporation revealed that this process is spontaneous and occurs as follows: once the cholesterol molecule has reached a nanotube opening, it irreversibly enters the nanotube (*Figure 2*). The first stage, namely the initial approach to the opening, takes 510 ps at 300 K and 280 ps at 1,000 K when the nanotube is prehydrated, i.e. initially contains water inside. At 1,000 K, due to accelerated motion, the cholesterol molecule had time to change its orientation from being directed with its hydroxyl group towards the nanotube to being directed outwards. The whole incorporation takes 770 ps at 300 K and 440 at 1,000 K. This gives an estimate of the activation energy in the order of 3.5-3.7 kcal/mol.

One may easily track down the beginning of the cholesterol being engulfed by looking at the potential energy of the cholesterol (*Figure 3*): it starts to decrease at the entry and reaches a plateau when the molecule gets trapped. A change in fluctuations of electrostatic energy in the cholesterol molecule indicates the completion of incorporation: the cholesterol molecule adopts more or less stable conformation and the fluctuations practically vanish, leaving opportunities to intensify at spikes related to rare conformational movements. On average, the only interactions which play a role and, correspondingly, change are Van der Waals interactions. Only they, not the electrostatic ones, change significantly. Although the cholesterol loses favorable Van der Waals interactions with water (there are less water molecules surrounding the cholesterol in the nanotube), more favorable attraction of the nanotube's wall prevails and thus makes overall potential well of ~ 30 kcal/mol.

When the nanotube is prehydrated the process of penetration generally takes more time at 300 K. This is explained by the initial absence of water inside the nanotube (although water molecules easily enter the nanotube from the free opening, as previously predicted from other MD simulations [44-47] and even earlier in experiments [48-50]). According to *Figure 3*, which is in the case of a prehydrated nanotube at 300 K, the commencement of entering is after 500 ps. For comparison, at 1,000 K it takes only 80 ps for the cholesterol to start entering the nanotube and approximately 250 ps to complete the whole process. However, at high temperature such as 1,000 K fast absorbing movements, resulting from the initial absence of water inside the nanotube, may cause deflection from the straight way to

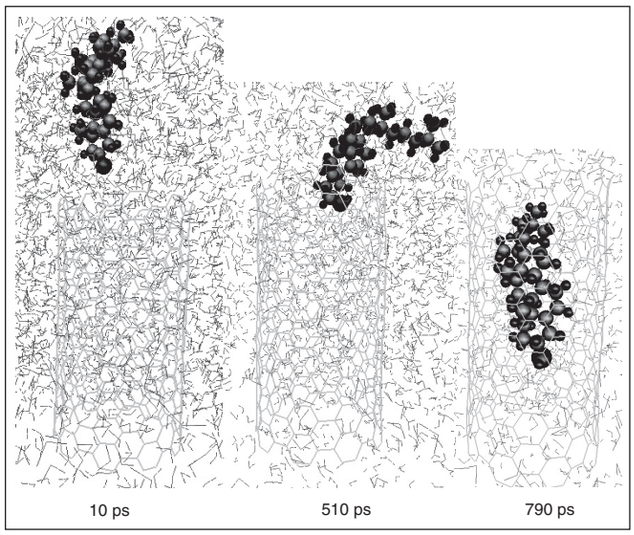

Figure 2 - Subsequent stages of cholesterol inclusion at 300 K. The nanotube is prehydrated.

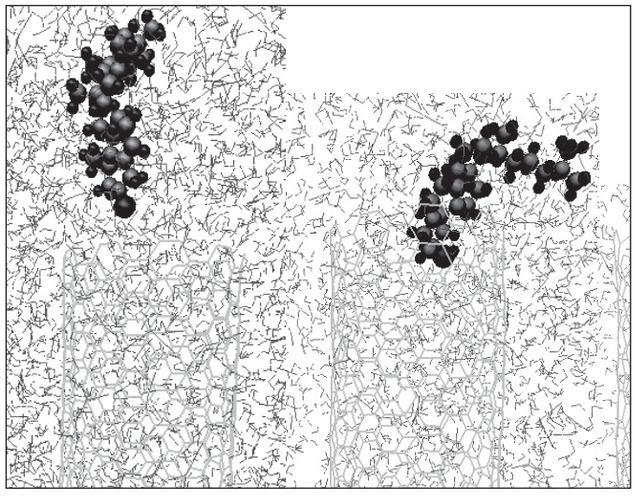

Figure 3 - Potential energy in the system. The energy is represented by the following terms: 1. interactions between the cholesterol's atoms, 2. term 1 plus interaction of the cholesterol with the water, 3. term 1 plus interaction of the cholesterol with the nanotube, 4. total potential energy of interaction of the cholesterol's atoms with the system's atoms.

the very opening of the nanotube. That is why in some simulations, approach from the outer wall of the nanotube was observed. After the cholesterol is adsorbed on the external surface, it takes quite some time to approach a nanotube's opening, losing thus favorable contacts with the wall, and try to penetrate again.

2. Interaction of the polypeptide with the nanotube

In our numerical experiments, self-assembly of a polyalanine and a carbon nanotube accompanied by formation of a structure in the form of polyalanine helix inside the nanotube was revealed. It was shown that at 300 K during the period of time in the order of 200 ps inclusion (*Figure 4*) of the polypeptide in alpha-helical conformation on the surface of the nanotube took place. When this occurred there was initially a distance of 30 Å between the peptide and the nantotube.

Further changes in the system may be traced by using the method of acceleration of over-barrier transitions at increased temperature. In such a way, the process of spontaneous penetration of the polyalanine into the nanotube was observed. Despite the fact that the benefit in energy in this case is much greater than at adsorption of the peptide

on the external surface of the nanotube, the transition of the peptide from the state of being outside the nanotube to the inside-the-nanotube state requires a certain energy barrier to be overcome as the adsorption energy of the polypeptide decreases when the peptide moves to the periphery of the nanotube.

The detailed picture of the self-assembly act of the structure under investigation is presented in *Figure 5*. Being on the external wall of the nanotube, for most of the time the peptide remains in the central part of the cavity. Occasionally one of the peptide's ends finds itself near the aperture. Gradually owing to fluctuations, the peptide molecule moves along the nanotube. Then an end of the peptide comes nearer to the hole and subsequently the whole molecule quickly enters the nanotube. This phase lasts 130 ps at 1,000 K. At 2,000 K the self-assembly goes with the same mechanism, however at higher temperature the process becomes more reversible. Because of this the time from the beginning until the completion of the embedding increases to 300 ps.

Duration of the formation of the configuration which is active for self-assembly at 1,000 K is 4.64 ns, at 2,000 K - 0.655 ns. This gives an estimate of the activation energy at about 7.8 kcal/mol. The expected time of the self-assembly at 300 K makes 43μs. It is significant that the considered process was simulated in a vacuum. For processes which take place in solvents the activation energy of the self-assembly should apparently be lower owing to the effect of solvation energy of the polypeptide and the nanotube.

3. Nanosyringe in action

The complex of the polypeptide and the capped nanotube considered above may be used for delivery of a peptide (or another molecule) through a biological membrane to a cell or separate compartment. A common task is to selectively deliver low-molecular synthetic molecules imitating the functioning of natural biomacromolecules and having a therapeutic potential. These constructions may also be applied to studying mechanisms of molecular recognition.

It is worth noting that the generation of such systems may soon give rise to a new trend – nanopharmacology. Molecular dynamics appear here as a tool for designing a functional molecular construction, thus enabling the necessary parameters of the device to be determined. As an example, a nanosyringe pushing a peptide out from the nanotube into bilayer membrane or water was simulated.

Eight swelling model Van der Waals spheres expelling the polyalanine out from the nanotube were taken as an active agent. The radius of the spheres increased to the values in the order of the radius of the nanotube, thus the rate of radius' augmentation made 0.25 and 0.5 Å/ps (extension time was 26 and 13 ps, respectively). Under such circumstances release took place at a huge rate of expulsion. In *Figures 6 and 7* a scenario of peptide ejection at such extreme parameters of the "shot" (maximum pressure in the nanotube was about 10^5 bar) is depicted. At the moment of "shot" the nanotube becomes a little deformed, but the deformations do not exceed its strength. After the termination of the process of peptide expelling the nanotube completely recovers the initial conformation during the time on the order of 3 ps.

In the course of the process considered above the polyalanine molecule experiences conformational changes. The initial helical conformation deformed most greatly at ejection of the polypeptide into a vacuum and least of all – into the membrane. Apparently, the environment plays a deforming and structure-forming role in this process. Conformational changes of the polypetide molecule are reduced with a decrease of the swelling rate, as seen in *Figure 8*. A little widening over conformations when ejecting the peptide into water may be related to additional strain arising at the entering of the hydrophobic molecule into water medium.

The present all-atom MD simulation confirmed the initial idea

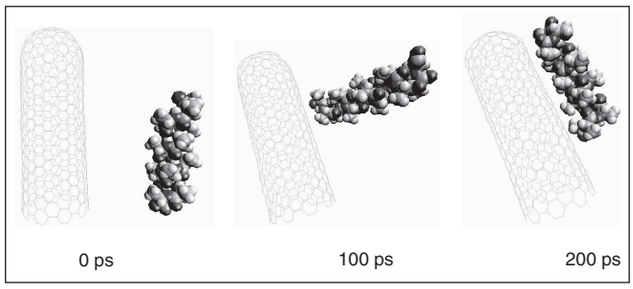

Figure 4 - Subsequent stages of attachment of the polyalanine to the external surface of the nanotube.

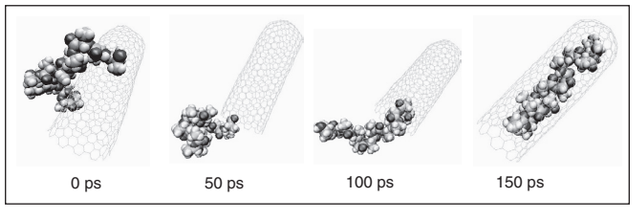

Figure 5 - Subsequent stages of the self-assembly of the peptide-nanotube complex at 1000 K.

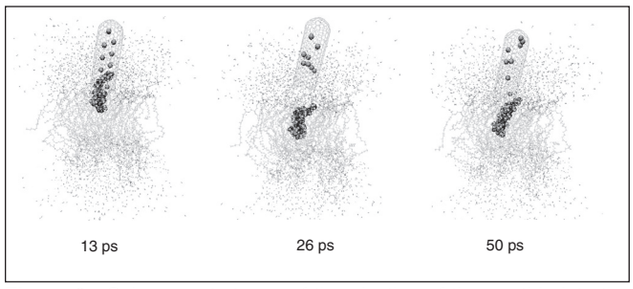

Figure 6 - Subsequent stages of the ejection of the peptide into the membrane.

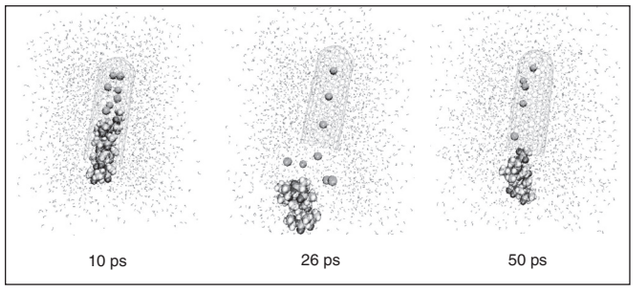

Figure 7 - Subsequent stages of the ejection of the peptide into water.

about applying carbon nanotubes as nanocontainers for biomolecules. Indeed, no matter whether a nanotube is prehydrated or not, it can easily accommodate small non-hydrophylic molecules. Another aspect of such nanocontainers consists in a logical development of nanotube-based devices such as the nanosyringe. Computer simulations, although in a little simplified model, corroborate the principle possibility of proper functioning. By having a molecule available that may be activated by light [1, 51], an experimental approach may be counted on.

In future, by tuning the nanotube (by adding functional groups) both selective inclusion and selectivity of nanotube's landing area on the cellular membrane may be achieved. The results of the current MD study give a powerful instrument of engineering of a real device to be invented.

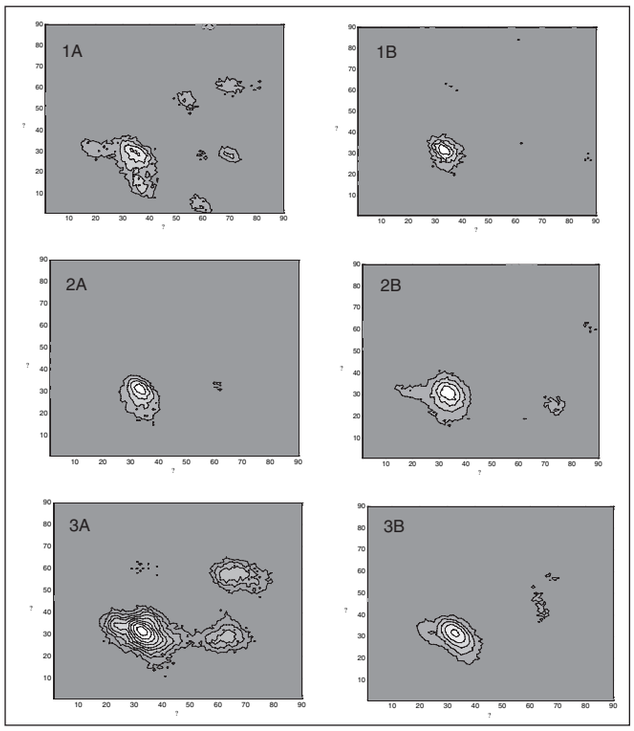

Figure 8 - Poincare sections for the polyalanine's φ and ψ angles averaged over all residues under extrusion. The extrusion was to the membrane (1), water (2) and vacuum (3) at the spheres' swelling times of 13 ps (A) and 26 ps (B).

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