Adsorption of the pyrene-functionalized single stranded oligonucleotides onto the SWNTs surface

M.V. Karachevtsev[1*], E. K. Apartsin[2], D. S. Novopashina[2]

[1] B. Verkin Institute for Low Temperature Physics and Engineering of the National Academy of Sciences of Ukraine, Nauky Ave., 47, Kharkiv-61103, Ukraine.

[2]Institute of Chemical Biology and Fundamental Medicine SB RAS, 8, Lavrentiev ave., Novosibirsk, 630090, Russia

E-mail: mkarachevtsev@ilt.kharkov.ua

Design and investigation of new hybrid functional nanomaterial for biological and medical application are novel rapidly developed scientific area at the interfaces between chemistry, physics, biology and medicine. Single walled carbon nanotube (SWNT) is the object of special interest in last decade as nanoplatform for biohybrids as instrument for molecular biology and nanomedicine. One of the applications of SWNT is their use for the drug delivery, for example, small interfering RNAs (siRNAs). Previously, it has been found that siRNAs can effectively inhibit the expression of target proteins. siRNA is a short double-stranded RNA with two free nucleotides at the 3'-end.

Direct immobilization of siRNA on a carbon nanotube leads to a decrease in the biological activity of siRNA, so to overcome this disadvantage, non-covalent immobilization of oligonucleotides on the SWNT's surface through flexible pyrene linker is used. Such immobilization is based on strong π-π stacking interactions between the SWNT's surface and pyrene, which is attached to siRNA covalently. Therefore, the main purpose of this work is to elucidate the process of formation of the hybrid of carbon nanotubes with pyrene-modified oligodeoxyribonucleotides as the subsequent formation of complex with siRNAs employing molecular dynamics method (MD) and DFT calculations.

MD simulation protocol:

- Charmm force field,
- NAMD program package,
- NPT ensemble,
- PyrPEG$_6$ Pyrd17 - 18 sodium ions, 30673 H_2O molecules, dimensions of water box - 86Å×48Å×45Å,
- SWNT-PyrPEG$_6$ Pyrd17 - 18 sodium ions, 30673 H_2O molecules, dimensions of water box - 178Å×51Å×110Å,
- Pyrd17/mdr1-LS - 54 sodium ions, 8332 H_2O molecules, dimensions of water box 57Å×60Å×178Å,
- SWNT- Pyrd17/mdr1-LS - 54 sodium ions, 30690 H_2O molecules, dimensions of water box 178Å×99Å×56Å

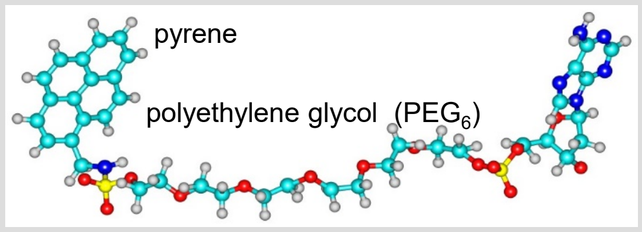


Fig. 1. Structure of flexible pyrene linker.

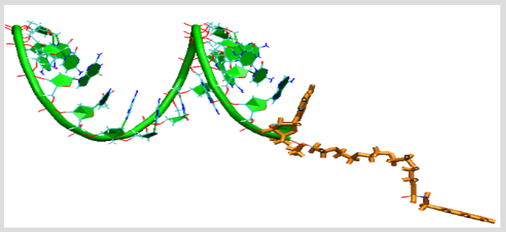
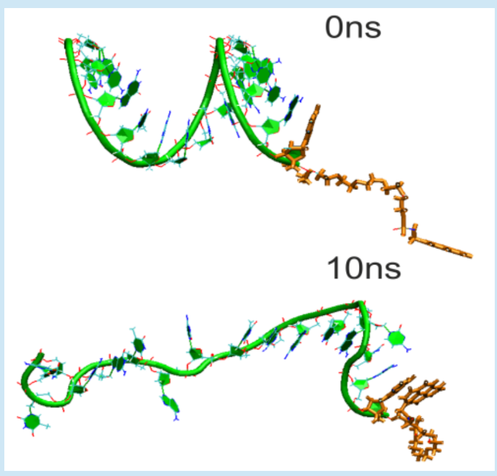


Fig. 2. Structure of siRNA (PyrPEG$_6$ Pyrd17) with flexible pyrene linker

Development of flexible pyrene-polyethylene glycol linker for the simulation of oligonucleotides adsorption on the SWNT surface



The equilibrium structure of PyrPEG$_6$ Pyrd17 after 10 ns of simulation is shown in Fig. 3. It follows from the figure that after 10 ns the helical structure of the oligonucleotide was partially broken due to thermal fluctuations in which only 7 bases in stacking with each other. As a result we obtained the elongated conformation of the oligonucleotide. In addition, after 3 ns between pyrene and adenine of the nearest nucleotide, a π-π stacking complex was formed. At that the PEG$_6$ formed the ring practically. We estimated the binding energy of the pyrene with adenine in the water environment which is about 10-15 kcal/mol.

Fig. 3. Snapshots of Pyrd17 with flexible pyrene linker in different simulation times: the initial orientation and after 10 ns.

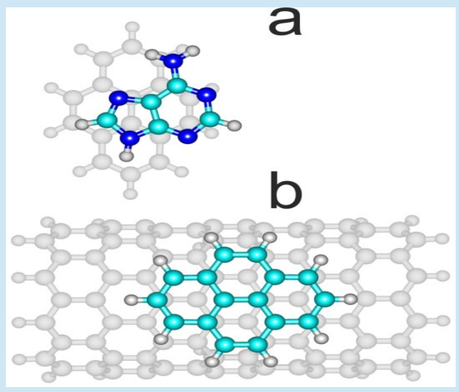


Fig. 4. Structure of the pyrene-adenine complex (a) and pyrene-SWNT (b) complexes.

Since the formation of the pyrene-adenine complex is important for the subsequent analysis of oligonucleotides adsorption through the pyrene anchor, this complex was calculated using the DFT method (M06-2X, 6-31++G** basis set). We analyzed the stacking pyrene-adenine complex by twisting one molecule around another to full turn with 30^0 step and calculated the interaction energy values in each point. As a result three most stable conformations were found with an interaction energy ranged with -11.8, -11.7 and -10.8 kcal/mol. The comparison of the obtained values of the interaction energies with the binding energy of pyrene with SWNT (-16.7 kcal/mol for the with 1.25 nm diameter of nanotube) obtained earlier showed that the binding of pyrene with the nanotube is stronger than pyrene with adenine by 3.9-4.9 kcal/mol.

Adsorption of the pyrene-functionalized single stranded oligonucleotides onto the SWNT surface: MD simulation.

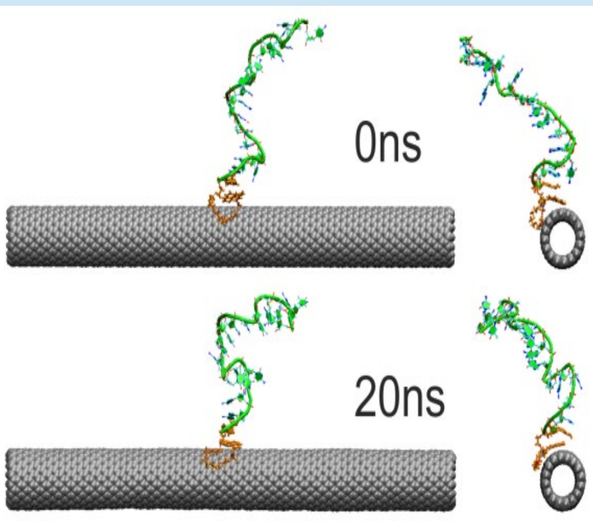


Fig. 5. Snapshots of the SWNT-PyrPEG$_6$ Pyrd17 hybrid with flexible pyrene linker in different simulation times: the initial orientation and after 20 ns.

To analyze the adsorption of PyrPEG$_6$ Pyrd17 on the nanotube, we placed an equilibrium structure of the oligonucleotide near the surface of the nanotube in a water box. As one of the variants of the arrangement of PyrPEG$_6$ Pyrd17 relative to the nanotube, an approximately perpendicular arrangement of the elongated oligonucleotide relative to the nanotube axis was selected, placing pyrene near the nanotube surface (Fig. 5). After that, the whole system was simulated for 20 ns. The structure of the SWNT-PyrPEG$_6$ Pyrd17 hybrid in the starting geometry and after 20 ns of simulation is shown in Fig. 5. Simulation showed that all this time the SWNT-PyrPEG$_6$ Pyrd17 hybrid is stable and their relative position at the end of the simulation is not very different from the starting one.

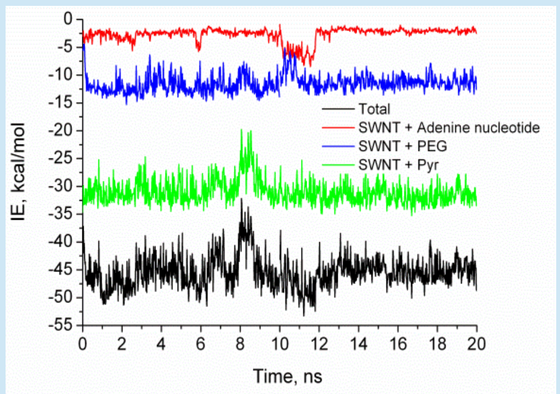


Fig. 6. Dependence of the interaction energy between the components of the SWNT-PyrPEG$_6$ Pyrd17 complex on the simulation time. Blue curve shows the energy of interaction between PEG and SWNT, green - between pyrene and SWNT, black - the total interaction energy between SWNT and PyrPEG$_6$ Pyrd17.

We calculated the dependence of the binding energy oligonucleotide with the nanotube surface on the simulation time (Fig. 6). The interaction energy of the oligonucleotide and SWNT reaches the stable value for short time due to close location of pyrene near SWNT surface. The main contribution to the interaction of the oligonucleotide with the nanotube surface (~45 kcal/mol) occurs due to the π-π stacking interaction between pyrene and SWNT (~ 30 kcal/mol). Note that pyrene, in addition to the interaction with SWNT, is in the stacking with the nearest nucleic base (adenine) with the energy value of ~10 kcal/mol. This stacking is maintained during the whole simulation time (Fig. 5). The stacking structure of SWNT-pyrene-adenine and self-stacking of nucleic bases inside the oligonucleotide stably maintain the strand near the nanotube surface, which makes it possible to hybridize with the complementary strand. For single pyrene, the energy barrier between SWNT hexagons is quite low and this circumstance provides the mobility of this molecule along the carbon surface as well as conjugated biomolecule with them.

Adsorption of double stranded oligonucleotides onto the SWNTs surface through the pyrene-functionalized one strand.

For analyzing of the adsorption of double stranded oligonucleotide on the SWNT surface we selected a duplex consist of PyrPEG$_6$ Pyrd17 and of 36-mer oligonucleotide (mdr1-LS) which has 15-mer oligomeric fragment with a sequence complementary to the first oligomer). This duplex, consisting of two strands of different lengths, was simulated for 10 ns in order to obtain an equilibrium structure of PyrPEG$_6$ Pyrd17/mdr1-LS in an aqeuous environment (Fig. 7). Note that although the starting structure loses a rigid conformation, nevertheless, the double stranded oligonucleotides keeps the duplex structure during all simulation time. By 10 ns of the simulation, one pair of hydrogen-bound bases is dissociated, but pyrene, unlike to simulation of single-stranded PyrPEG$_6$ d(P)$_{17}$, does not form a stacking structure with the nearest adenine of this strand or guanine of the neighbor strand.

Pyrd17/mdr1-LS:

5′- Pyrd(AACCGTGGTCATGCTCC)

5′- r(GGCUUGACAAGUUGUAUAUGGGGAGCAUGACCACGG)

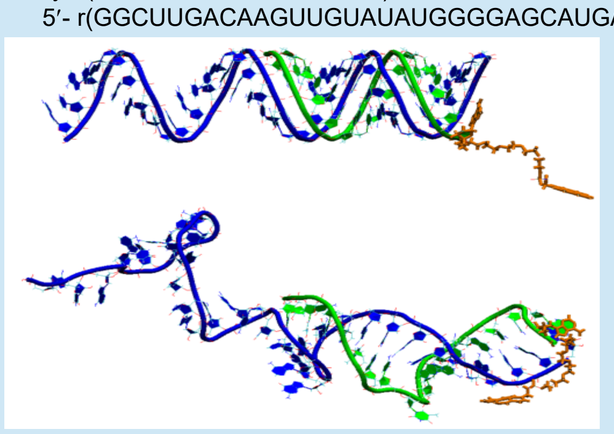


Fig. 7. Structure of the PyrPEG$_6$ Pyrd17/mdr1-LS duplex with a flexible linker before and after 10 ns of simulation.

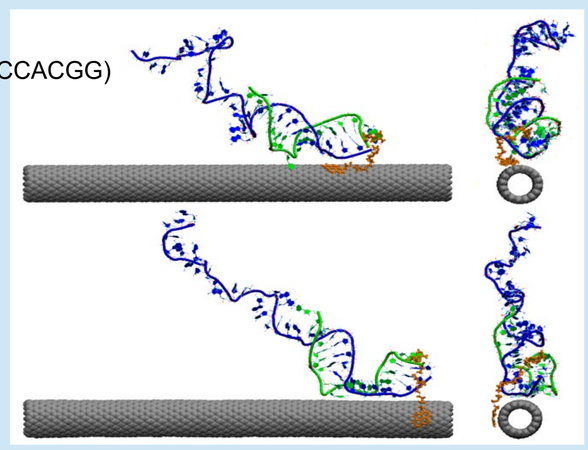


Fig. 8. Structure of the SWNT - PyrPEG$_6$ Pyrd17/mdr1-LS complex in the first variant of the relative arrangement in starting simulation time and after 35 ns of simulation.

The structure of the complex PyrPEG$_6$ Pyrd17/mdr1-LS:SWNT after 30 ns of simulation is presented in Fig. 8 (lower snapshot). The simulation showed that all the time this formed complex remains stable and pyrene plays its role of the reliable anchor on the nanotube surface.

Figure 9 shows the dependences of the interaction energy of each strands of the duplex with SWNT on the simulation time. It follows from this Figure that PyrPEG$_6$ Pyrd17 rapidly increases its binding energy with the nanotube. The second strand (mdr1-LS) begins to interact with the nanotube only after 16 ns of simulation, mainly due to the interaction of sugar-phosphate backbone and back-side groups of the first ten nitrogenous bases. By 35 ns, this energy reaches a value of about -50 kcal/mol. Note, that this strand has no bases stacked to the nanotube. We indicate that the pyrene frequently changes of its location on the nanotube surface during simulation due to the small value of the energy barrier between the hexagon of the SWNT.

Thus we can concluded that PyrPEG$_6$ Pyrd17/mdr1-LS keeps its duplex structure near the nanotube surface during all time of simulation. The interaction energy between them depends on the location of the oligonucleotide relative to the nanotube. The anchor molecule of pyrene plays an important role in oligonucleotide retention near the nanotube, especially at the initial stage. Rapid adsorption of pyrene to the surface of the nanotube makes it possible to exclude the stacking structures of this duplex with the nearest bases.

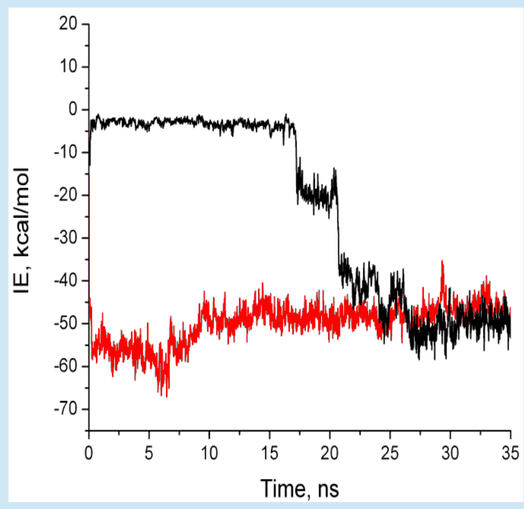


Fig. 9. Dependence of the interaction energy between the components of the PyrPEG$_6$ Pyrd17/mdr1-LS : SWNT complex in the first variant oligomer-nanotube arrangement on the simulation time. Red color shows the interaction energy between PyrPEG$_6$ Pyrd17 and SWNT, black – between mdr1-LS and SWNT.

Conclusions

1. Molecular Dynamics simulations has shown that pyrene, as an interface molecule between the siRNA and the SWNT, quite reliably holds single-stranded and double-stranded oligonucleotides (20-30 nucleotides) near the surface of the SWNT due to π-π stacking interaction between pyrene and the nanotube. Note, that siRNA and flexible pyrene linker allows to form a stacking dimer between the pyrene and the nearest nitrogen base. However, this dimer does not prevent the pyrene from adsorbing on the surface of the nanotube, because the interaction energy between the SWNT and pyrene (~ -15 kcal/mol) exceeds the interaction energy between pyrene and nearest adenine (less than -13 kcal/mol).
2. It is shown that, in contrast to a single-stranded oligomer, the duplex of siRNA can be stably immobilized near a nanotube with keeping structure by means of the pyrene linker attached at the duplex end. The pyrene anchor plays an important role in holding the duplex near the nanotube as sufficiently rapid adsorption of pyrene on the surface of the SWNT and rigid structure of the duplex prevents the formation of other contacts of siRNA with the nanotube.