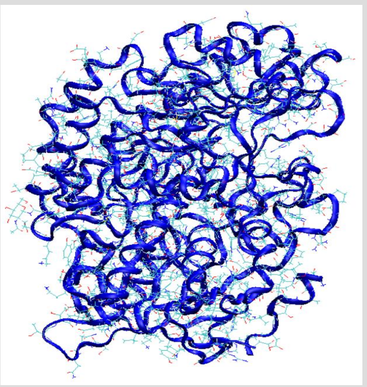
# Immobilization of glucose oxidase on graphene using 1-pyrenebutanoic acid succinimide ester: molecular dynamics study

**M.V. Karachevtsev[1*], V. A. Karachevtsev[1]**
*e-mail: mkarachevtsev@ilt.kharkov.ua*
*[1] B. Verkin Institute for Low Temperature Physics and Engineering of the National Academy of Sciences of Ukraine, Nauky Ave., 47, Kharkiv-61103, Ukraine.*

In an elaboration of biological sensors involving graphene one of the important problems that needs to be solved is related to the immobilization of the recognizing molecule on the carbon surface. As enzymes are often exploited as possible recognition elements in biosensors, their immobilization on the nanotube surface needs to be investigated in detail. The simple method of enzyme adsorption using direct immobilization on graphene can not be applied as its interaction with the carbon surface significantly decreases the enzyme activity.

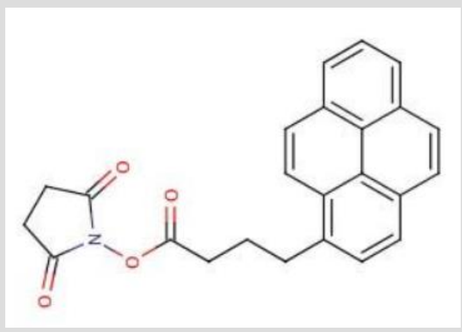
One of the real ways to solve this problem is related to the use of molecular interfaces. Earlier an effective noncovalent way to functionalize carbon nanotube by organic molecules was suggested a bifunctional molecule containing succinylimide ester and a pyrene moiety to bind proteins to the nanotube surface. Pyrene attaches to the nanotube surface employing the π-π stacking and hydrophobic interactions and does not significantly disturb the electronic structure of the nanotube.



**Simulation Protocol:**

- Charmm force field Graphene sheet of 120Å×124Å (5682 carbon atoms and 212 hydrogen atoms)
- The structure of the glucose oxidase molecule was obtained from the protein database
- Constant temperature and pressure (303K, 1 atm)
- Periodic boundary conditions

Fig. 1. Structure of glucose oxidase.



The parameters (i.e., the force constants and the equilibrium structure parameters concerning the bond distances, the angles, and the dihedral angles) for the atoms of the PSE molecule were obtained based on the results of quantum-chemical calculations performed at the B3LYP/6-31++G(d) level of theory.

Fig. 2. Structure of 1-pyrenebutanoic acid succinimide ester

## Adsorption of GOX on graphene in aqueous environment

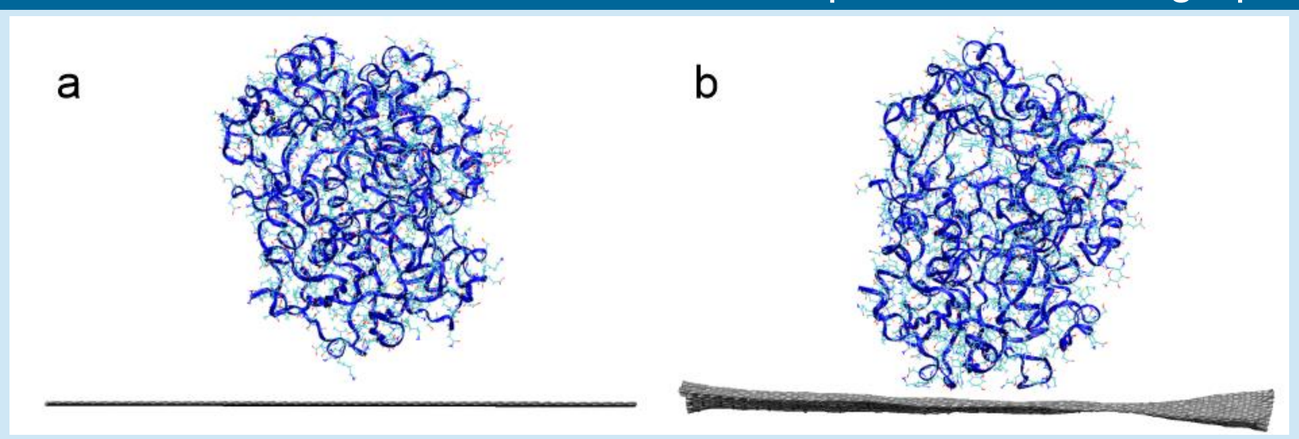



Fig. 3. The structure of the GOX complex with graphene before (a) and after 20 ns of simulation (b).

To evaluate the effect of the molecular interface on the interaction of GOX with graphene, we first performed MD simulations of the direct adsorption of this enzyme on graphene in aqueous environment. The simulation showed that at the very beginning, the GOX molecule approaches graphene and the interaction between them increases, as the number of amino acids contacts directly with carbon atoms is enhanced. The hybrid obtained remains quite stable throughout all time simulation (20 ns).

The dependence of the interaction energies between GOX and graphene on the simulation time is shown in Fig. 3. It can be seen that the interaction energy reaches values of about 55 kcal/mol to 4 ns, a significant increase in energy up to is observed. This increase is due to the fact that more amino acids begin to interact with graphene. The further increase in the interaction energy is slower, so after 16 ns the system stabilizes and the interaction energy is ≈110 kcal/mol.

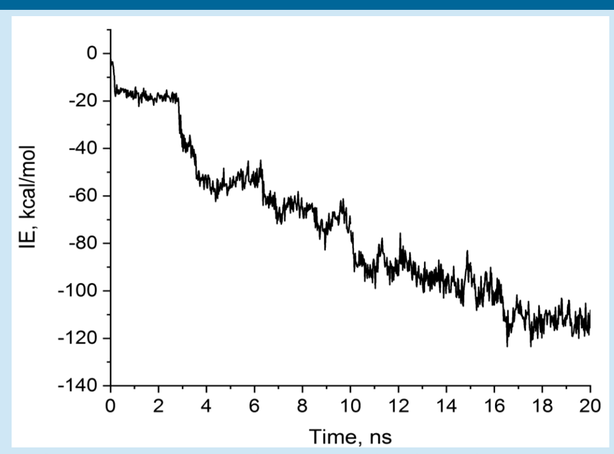
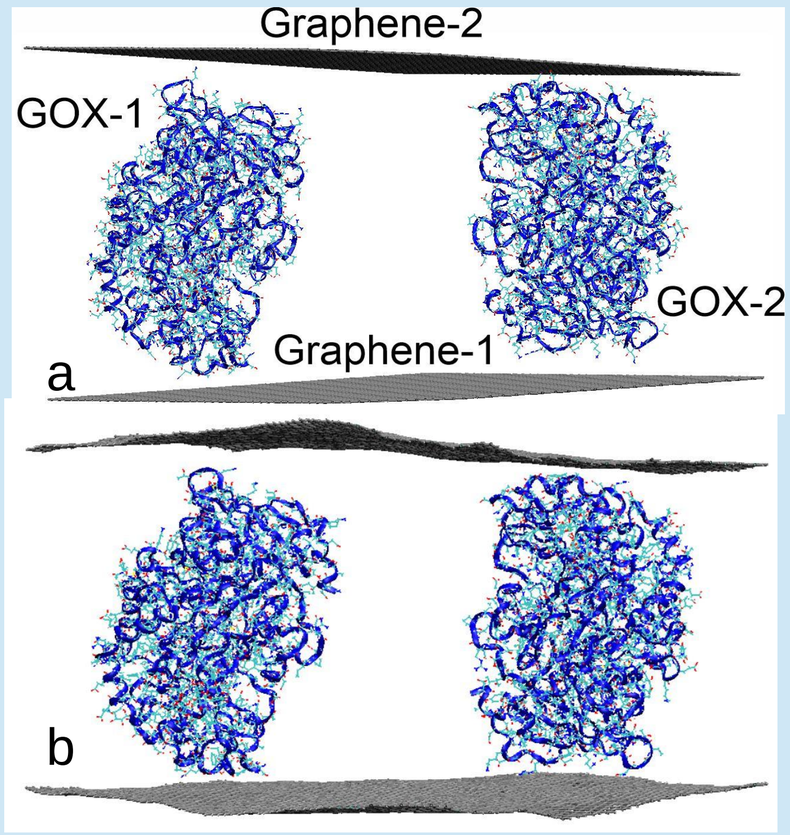



Fig. 4. Dependence of the interaction energies between the components of the GOX-graphene complex

Thus, the MD simulation showed that the GOX localized on the graphene surface stably throughout the simulation with the essential value of the interaction energy.

## MD simulation of 3D nanobiostructure formed by two GOXs located between two graphene sheets




We also simulated a more complex nanobiostructure formed by two GOXs located between two graphene sheets in aqueous environment. The structure of this nanobiostructure in starting and finish configuration are shown in Fig. 5 and 6, respectively. The simulation showed that the resulting nanobiostructure is quite stable. The interaction between graphene sheets and enzymes stabilizes the structure of the formed complex, the graphene sheets do not approach each other, and the enzymes do not move along the sheet. It should be noted that the structure of graphene sheets is changed during simulation, starting from flat structure and then is curved due to the temperature fluctuations of the carbon atom positions in sites. GOX molecules and graphene sheets interact through the contacts of amino acids located on the enzyme shell with graphene. The GOX-2 enzyme almost does not interact with the second graphene sheet, most likely due to the large distance between the enzyme and this graphene sheet.

Fig. 5. The structure of the complex of two GOXs between two graphene sheets before (a) and after 5 ns of simulation (b)

A sufficiently high energy of interaction between the GOX and the carbon surface can cause deformation of the space around the coenzyme, which leads to a decrease in the enzymatic activity of the redox reaction. To prevent such a decrease, it is proposed to use a molecular interface that prevents direct contact of the enzyme with the carbon surface.

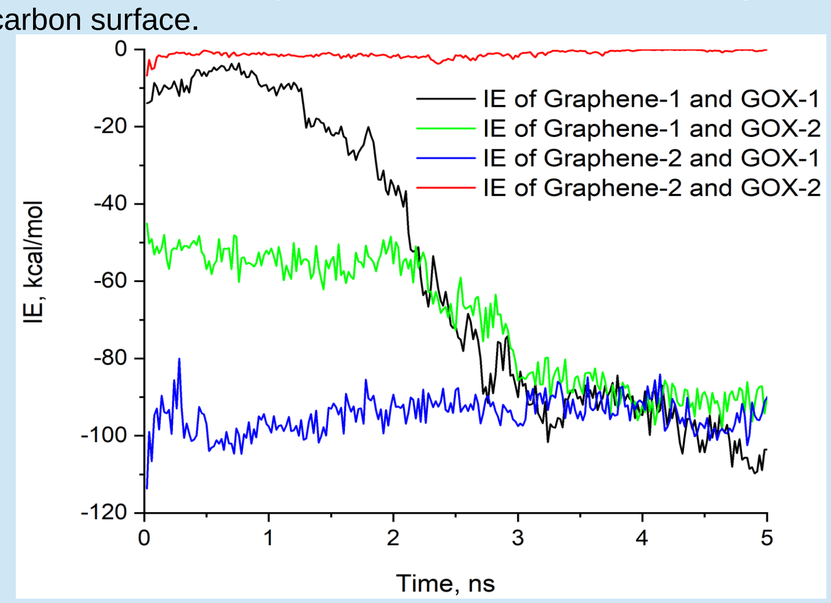



Fig. 6. Dependence of the interaction energy of GOX-1 and GOX-2 molecule with each graphene sheet.

## The influence of the molecular interface on the interaction between GOX and graphene

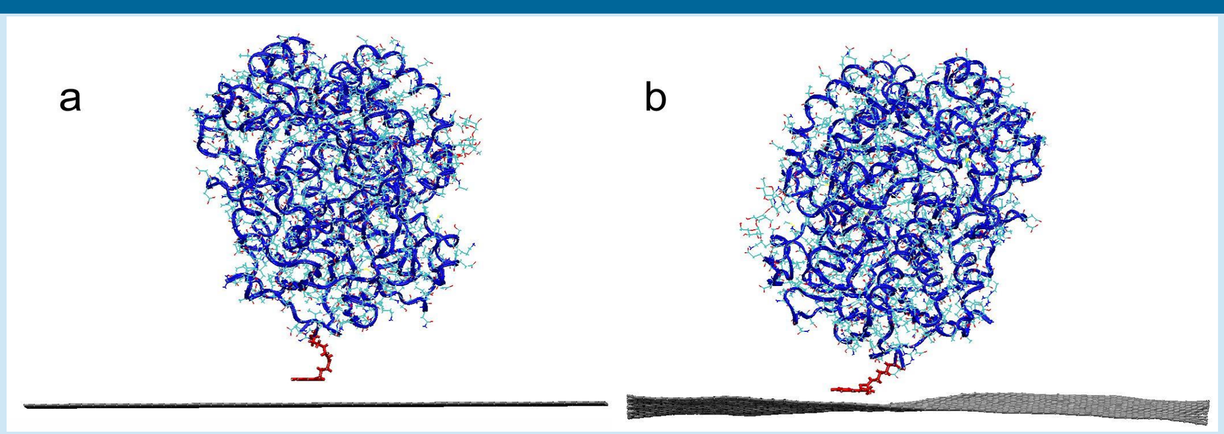



Fig. 6. The structure of the the graphene-PSE-GOX hybrid before (a) and after 20 ns of simulation (b) using the PSE molecular interface.

We simulated the adsorption of GOX to graphene using the PSE molecular interface. PSE is chemically bound to the GOX via the amino group of one of the amino acids. Then the pyrene fragment of the PSE molecule is located near the graphene surface.

In this arrangement, the flat pyrene molecule was placed in parallel with the graphene, and the other fragment of the PSE molecule with GOX was directed away from graphene.

In the graphene-PSE-GOX complex, the interaction energy between graphene and GOX with PSE ranges from -27 to -32 kcal/mol. This energy is mainly determined by the contribution of the interaction energy of pyrene with graphene. This value is essentially lower than the interaction energy of GOX and graphene without the interface. MD simulation also shows that only one PSE molecule used as a linker between graphene and GOX is enough to keep GOX near the carbon surface in the water surrounding and prevent the strong interaction between graphene and GOX.

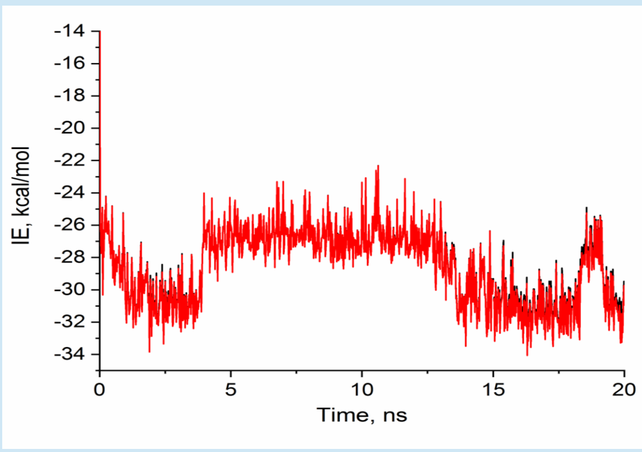



Fig. 7. Dependence of the interaction energies between the components of the graphene-PSE-GOX hybrid on the simulation time.

## Conclusions

- Using MD simulation the structure of the graphene-GOX complex is obtained and the interaction energy between the enzyme and the graphene surface was obtained, which reaches 110 kcal/mol. This energy value almost doubles when the enzyme is placed between two graphene sheets.
- To prevent the influence of the graphene surface on the functional properties of the adsorbed enzyme, a molecular interface PSE between them was proposed. The energy of interaction between graphene and GOX through the PSE interface is about -30 kcal/mol, which is significantly lower than in direct contact.
- Thus, molecular dynamics simulation showed that the PSE molecule is able to effectively retain the GOX with the carbon surface near the graphene surface and protect the enzyme molecule from deformation that occurs when interacting directly.