31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Molecular Mechanism for Conformation Mobility of the Active Center of Glucose Oxidase Adsorbed on Single Wall Carbon Nanotubes

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Abstract—**A critical issue in bioelectrochemical applications, that use electrodes modified by nanomaterials, like enzyme sensor modified by Single Wall Carbon Nanotubes (SWCNTs), is to ensure high activity of the active center of an immobilized enzyme protein. Since Flavin Adenine Dinucleotide (FAD) along with other amino residues, including His559, Glu412 and His516, constitute the active center of the catalytic site conformation of which could determine the activity of enzyme, it is important to understand the molecular mechanism of their mobility and the potential impact on the catalytic activity while GOx is immobilized on SWCNTs. However, this dynamic mechanism still remains blurry at the atomic level due to the active center being embedded in the apo-GOx and the limitations of appropriate experimental methods. The molecular dynamics (MD) simulation, as a successful approach for exploring some interaction details between protein and nanomaterials, was performed to investigate the mobility mechanism of the active center and the consequence for the possible change of catalytic activity in this study. The trajectory and bond distance clearly indicate that the adsorption of GOx onto SWCNTs with different orientations bring observable different interaction properties in the conformational mobility in active center. These results would help us understand some substantial factors for the activity of biomacromolecule while immobilized on nanomaterials.**

I. Introduction

GLUCOSE oxidase (GOx) electrodes have been extensively studied as a foundation for constructing biosensors, biomedical devices, enzymatic bioreactors and biofuel cells [1-6]. The most critical challenge in these applications is to immobilize the GOx so that it retains its enzymatic activity and permits fast and efficient electron transfer from the catalytic center to the electrode [7-10]. In order to achieve this, suitable electrode materials such as nanomaterials and special techniques for immobilizing enzymes on the electrode surface have been developed. Carbon nanotubes were first introduced by Iijima in 1991 [11] and they have come to be regarded as being a very attractive nanomaterial for a wide range of applications [5-6, 12]. Many experiments have been carried out to exploit the unique properties of SWCNTs that can lead to the preservation of catalytic activity and to the achievement of direct electron transfer with the redox active center of the adsorbed oxidoreductase in SWCNT-modified electrodes [5, 13-14]. It had been found that GOx complex and FAD coenzymes can spontaneously adsorb onto annealed carbon nanotubes with an armchair chirality to improve their bioelectrochemical performance respectively while these complexes are cast onto glassy carbon electrodes (GCEs) [5, 13-14]. At the same time, the result of cyclic voltammogram experiment showed that the peak current of intact GOx on a SWCNT-modified GCE is less than that of the electroactive FAD directly on SWCNT-modified GCE [13], and implied that structure of intact GOx and the conformation of active site bring apparent impaction on its catalytic activity. To make further clear relevant molecular details for this point, our group performed molecular dynamics simulation, which can provide a convenient and excellent semi-theoretical platform for estimating broad interactions between biomolecules and inorganic materials on the atomic level [15-20], to investigate the dynamic mechanism of the conformational mobility of a FAD coenzyme under the interaction between intact GOx and the sidewall of a metallic SWCNT with multiple adsorption orientations of the protein [18], and found that the driving force of conformational change of the FAD coenzyme originates from the direct interaction energy with water, not from that with SWCNT, but the effect of the interaction with SWCNT on conformational change could be attributed to the change in the water distribution caused by the hydrophobicity of the SWNCT. Since the FAD coenzyme, specially isoalloxazine, plays a key role on the catalytic activity for beta-D-glucose, the previous work [18] seem to be helpful for understanding the dynamics characteristics of the catalytic activity of intact GOx in molecular details while interacting

Manuscript received April 7, 2009. (Write the date on which you submitted your paper for review.) This work was supported in part by the Program for New Century Excellent Talents in University of China under Grant NCET-06-0527, Nature Science Foundation of Zhejiang Province of China under Grant Z1080300 and National Nature Science Foundation of China under Grant 30700153.

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with SWCNT. However, those reports from Wohlfahrt et al. and Michael et al. showed that FAD coenzyme associated with those auxiliary catalytic residues, containing His559, Glu412 and His516, to constitute the intact active center of the catalytic site. In those, Glu412 bound to His559, modulated powerfully its catalytic activity by affecting all the rate constants in the reductive and the oxidative half-reaction of the catalytic cycle, meanwhile, His516 was the catalytic base in the oxidative and the reductive part of the catalytic cycle [21,22]. From these cases, apparently, that the conformation of the active center, representing the active structure of redox site of GOx in order to recognize what are about the change of activity with GOx complex adsorbed on SWCNTs, it would be worthy of exploring the conformational mobility mechanism of intact active center, representing the active structure of redox site of GOx, to recognize further details in atom level about the interact mechanism between protein and nanomaterials while GOx complex is non-covalently adsorbed on SWCNTs.

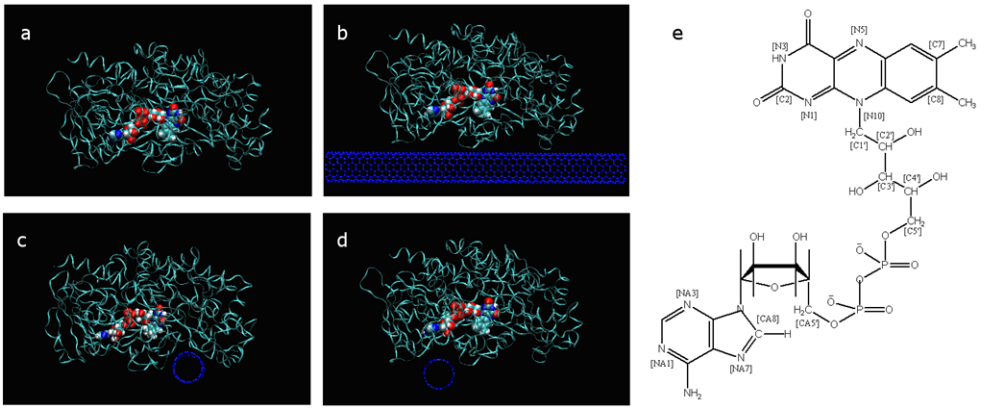


Fig. 1. (a) System A with a water box size of 99.5×69.5×79.7 Å3; (b) system B with a water box size of 124.0×91.6×82.5 Å3, in which SWCNT covers two pockets; (c) system C with a water box size of 124.1×88.0×98.0 Å3, in which SWCNT is close to the primary pocket; (d) system D with a water box size of 122.7×92.0×101.9 Å3, in which SWCNT is close to the secondary pocket. In these figures, water molecules are not displayed for clarity. (e) The chemical structural of formula of FAD tagged with an atom identifier.

Based on previous research performed by our group [16-19], in this study, an MD simulation with multiple adsorption orientations of the protein was conducted to investigate the dynamic mechanism of the conformational mobility of intact active center under the interaction between intact GOx and the sidewall of a metallic SWCNT, and to evaluate the consequent catalytic activity. Furthermore, this investigation could help us to make clear some critical issues about the immobilization of enzyme with SWCNTs in bioelectrochemical applications.

II. MATERIAL AND METHOD

Since material and method of the investigation was similar with that previous research performed by our group [18], corresponding information should be presented briefly.

The X-ray structure coordinates of Aspergillus niger GOx (PDB entry 1GAL) (EC 1.1.3.4) was refined to a resolution of 2.3 Å in ref. [23]. A monomer from the GOx homodimer was used in this study, and the formula of FAD is shown in Fig. 1e. SWCNTs with (7, 7) armchair chiralities were generated using the program Tubegen [24], Because of representing the inherent characteristics of high surface area, the sidewall of which was taken as the important sites to be investigated.

There are two pockets closer to the FAD coenzyme than other surface sites of GOx. In this paper, the one close to isoalloxazine is denoted as the primary pocket since it is where the catalysis process occurs; the other pocket, being close to the adenine of FAD and the auxiliary channel for relay electrons generated at the catalysis active site [2,25], is referred to as the secondary pocket. The shorter distance of the redox active cofactor of the GOx can make more profit of Direct Electron Transfer by tunnelling effect. So far, these two pockets were used as key sites for building three different adsorption orientations to examine the effect of intact active center that is influenced by the interaction between GOx and SWCNTs on the conformational mobility. In order to compare the effect of absorption on the sidewalls of the SWCNTs, a system of GOx without SWCNTs was generated as base system and denoted as system A. Based on the location, state and orientation of FAD in apo-GOx, the other three systems were generated and denoted as systems B, C and D, and all four systems are shown in Fig. 1a.

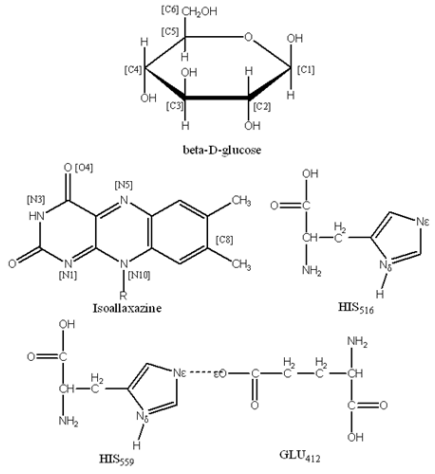


Fig.2. Chemical structure of formula of β-D-glucose; Isoalloxazine, His516, His559 and Glu412 in the active site modal

The energetically most favourable system, found in reference [21,22], was the one with hydrogen atoms at His559 Nε and at His516 Nδ. The carboxy oxygen Oε1 of Glu412 is located at a distance of 2.8 Å to His559 Nε, facilitating the formation of a hydrogen bond to H (Nε). The structure of those residues and formulas was shown in Fig. 2. Therefore, the target conformation concerning with those residues was taken as key focus to be investigation.

Simulations were performed in the following manner. First, 100 energy minimization iterations were performed, followed by equilibration of system A in an aqueous solution. A SWCNT with a length of 97.2 Å. was long enough for ignoring any influence of the edge effect for the adsorption of GOx on SWCNTs with different starting orientations. This was done to take the effects of the periodical conditions and the dimensions of GOx into consideration. The 200-ps equilibrated GOx without SWCNTs was used as the initial state for systems B, C and D. Then, 100 iterations of energy minimization were performed for all three systems.

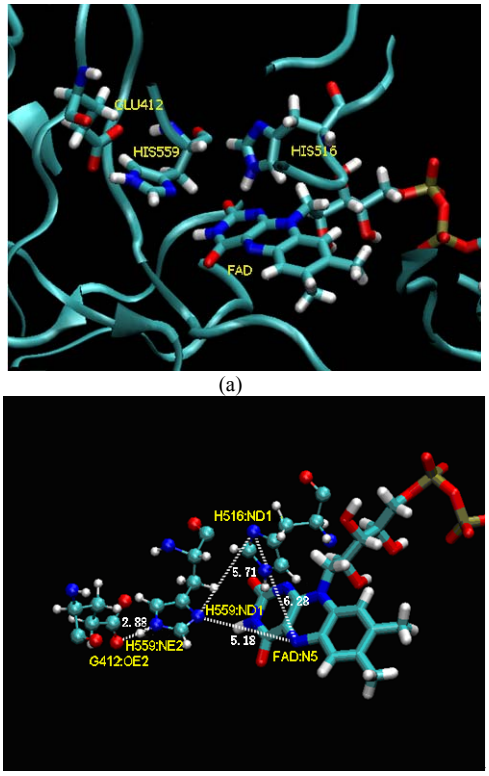


Fig. 3. Those conformations of catalysis site including FAD, His516, His559 and Glu412. (a) The catalysis site around beta-sheet; (b) Those key atoms for catalysis and their distances.

All four systems were solvated in a box of TIP3 water. The water box should be large enough to contain all the systems. All systems then underwent 2 ns MD runs using the program NAMD [26]. The force field parameters for the SWCNT and the apo-GOx were taken from CHARMM27 [27], and the force field of the cofactor FAD was empirically constructed from a combination of FMN and ATP in GROMACS [28]. The parameters for the Lennard–Jones potential for the cross interactions between non-bonded atoms were obtained from the Lorentz–Berthelot combining rule [29]. All the geometric structures of the different systems were visualized by VMD [30]. All simulations were performed with a time step of 2 fs, and a cut-off was set with a switching function starting at a distance 10 Å and reaching zero at 12 Å. A particle mesh Ewald (PME) summation was used to calculate the long-range electrostatic interactions, with a cut-off distance of 12 Å for the separation of the direct and reciprocal space. During the MD simulations, the Langevin method to ensure a constant temperature of 310 K and a constant pressure of 101.3 kPa. Periodic boundary conditions were applied for all the simulations. The interaction and potential energies were calculated and analyzed using the tools included in the VMD environment.

III. RESULTS AND DISSCUSION

Taking into consideration that relationship between conformation and function, to study the impact on the catalytic activity of GOx led by SWCNT adsorption, it is essential to discover the conformation dynamics of the active site, which including FAD, His516, His559 and Glu412. Like FAD, those auxiliary catalytic residues, including His516, His559 and Glu412, also play fairly important role in binding and making protonation with β-D-glucose [21,22]. Therefore, according to results from published reference [21,22], Since those distances among atom sites related to catalytic process of GOx could be regarded as proper parameters to present the catalytic conformation, for clarity and simplicity, those distances and their dynamics would be investigate to discover the effect on the catalytic activity from SWCNT adsorption..

The optimized distance between H(C1) of β-D-glucose and N5 of the isoalloxazine of FAD is 2.4 Å and H(O1) of the substrate is located 1.8 Å away from the unprotonated Nδ of His559. Being deduced from the geometry and the optimized distance [21,22], it seems most reasonable that H(C1) of β-D-glucose is transferred to N5 of FAD, and H(O1) may be transferred to Nδ of His559, which thereby becomes positively charged, thereafter, the proton at Nε of His559 may be transferred to the Oε1 of Glu412. This potential mechanism of the enzymatic reaction differs from the corresponding two-electron reductions of free flavins with a transfer of hydrogen protons to N1 and N5 of the isoalloxazine ring [22]. However, a proton transfer to N1 of the FAD of GOx is unlikely because N1 is located at the opposite side of the estimated substrate position and it is surrounded by other residues. At same time, a proton transfer to Nε of His516 seems less probable since this His is not activated by a Glu or Asp residues, but the calculations from reference [22], indicated that His516 is of central importance for GOx even though a proton transfer to His559 leads to more stable products. It seems more likely that the conserved His516 might be involved in the common step of the enzymatic reactions, which is the oxidative half reaction of the FAD.

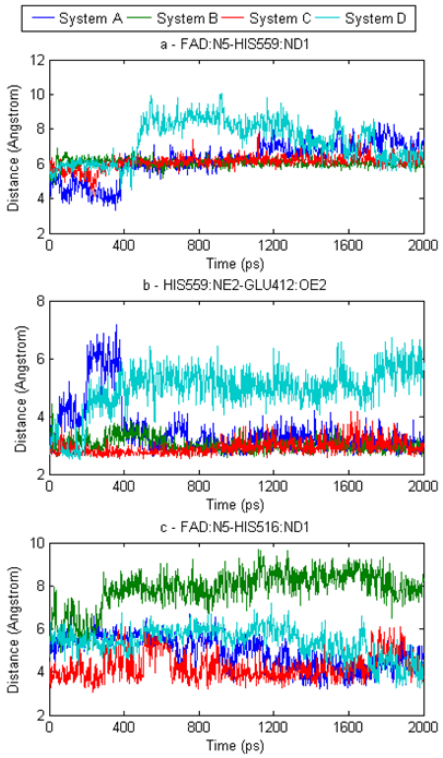
In the PDB file of this MD simulation, Nδ of His559 was

denoted as His516:ND1, Nδ of His516 as His516:ND1, and the Oε1 of Glu412 as Glu412:OE2. In terms of the active conformation of catalytic site resulted revealed in the published reference, there are the optimized distances, among His516:ND1, His516:ND1 and Glu412:OE2, to keep the catalytic site active. Therefore, as shown in Fig. 3, those distances, including His516:ND1-FAD:N5, His559:ND1-FAD:N5, His516:ND1-His559:ND1 and His559:NE2-Glu412:OE2, were used to approximate the active conformation of GOx.

Generally speaking, the GOx in system A, being alone in the aqueous, can keep its activity. As the interaction of them with water molecules, the distances facilitating the catalytic activity should be changing dynamically. But when system A achieves equilibrium, the fluctuation of distance could come into relatively stable. It should need a benchmark of the active center to evaluate the effect of SWCNT absorption on the conformation of the catalytic site, at same time to avoid those different meanings from different simulation conditions. And in system A, it may be reasonable that GOx in the aqueous without SWCNT adsorbing can maintain its nature. Also, it was found that the incremental distance among those atom sites of active center could make negative effect on the formation of hydrogen bonds that concern with the protonation between glucose molecule and those atom sites, eventually maybe lead catalytic site to denaturation. In contrast, the shorter distance should be more conducive to forming hydrogen bonds than the former, and more helpful for the catalytic process. Those conditions can be taken as some simple criterions to judge the potential effect of SWCNT absorption on the activity of catalytic site.

Considering that H(C1) of β-D-glucose is transferred to N5 of FAD, that H(O1) may be transferred to Nδ of His559, and that the distance between H(C1) and H(O1) almost is equal to constant, as shown in Fig. 4(a), it could be observed that the distance of His559:ND1-FAD:N5 in system B and system C keep very stable state, ~6 Angstrom, all similar with that in system A during the whole MD simulation. However, that in system D showed large fluctuation before 1.4ns and similar fluctuation trends like that in system A, with that in system A after 1.4ns, central value of which around 7 Angstrom. So for the distance of His559:ND1-FAD:N5, there should be no notable differences happen in all systems with SWCNT interaction.

Under the certain distance between His559:NE2 and Glu412:OE2, Glu412 bound to His559 through hydrogen bond, modulates powerfully its catalytic activity by affecting all the rate constants in the reductive and the oxidative half-reaction of the catalytic cycle. At some degree, the distance of His559:NE2-Glu412:OE2 could be taken as crucial parameter to evaluate the activity of catalytic reaction. As shown in Fig. 4(b), the fluctuation trends of His559:NE2-Glu412:OE2 demonstrate similarity among system A, system B and system C after 0.8ns, central value of which around 3 Angstrom. At the same time, there should be remarkable difference between the fluctuation trend in system D and those in the other three systems. In terms of distance of His559:NE2-Glu412:OE2, it seems more likely that the catalytic activity in system D became into denatured state. Since His516:ND1 and FAD:N5 all concern with the catalytic point in the oxidative and the reductive part of the reaction cycle, the fluctuation trends of His516:ND1- FAD:N5 also was used to estimate potential change of the catalytic activity. As Fig shown in 4(c), That fluctuation trends show similarity among system A, system C and the system D after 1.4ns, central value of which around 5 Angstrom., And for the fluctuation trends of the distance, there should be no distinct difference among in system A, C and D.. At the same time, it might happen apparently that the denaturation of GOx in system B.



Fig, 4. Trends of interact distance among those key atom in catalysis site during 2ns MD simulation.

By the above analysis and results of MD simulation, for catalytic conformation, system D and system B seemed more likely that denaturation of the catalytic site occur, at the same time, system C did not. In terms of some results provided in ref [18], the conformational mobility of active center primarily might be attribute to that of FAD, and secondarily to that of apo-GOx. Even for System C, due to the being of SWCNT could block the free access of the catalytic substrate, β-D-glucose, to the catalytic center, denaturation of GOx probably occurs consequently [18].

From this research, it could be found that SWCNT adsorption bring out observable effects for conformation mobility, enlarging the bond distance of those key active sites. Since the catalytic activity was associated with the bond distance of active site [21, 22], it should be reasonable to note

that the activity of catalytic center may take worse, even be denatured at some adsorption orientations. At one hand, as we all known, the present metallic SWCNT can raise the performance of Direct Electron Transfer (DET) observably in bioelectrochemical applications [7-10]. It would be likely to bring negative effect on the enzymatic activity with some adsorption orientations at other hand. Moreover, there may be potential difference between the results of published experiment and that in this work. So, it need further research to find that detailed information about the factors making that difference. Whatever, those results could give new insight into the molecular mechanism of denaturation of GOx or other imaginations under the interaction with SWCNT at different adsorption site.

IV. CONCLUSION

The more precise MD simulation along with beta-D-glucose should be performed to investigate the more detailed and practical process mechanism of the nanomaterial modified GOx electrode in the future. The ionic strength and the pH all play crucial role in fabricating bioelectrochemical sensors and devices, so those should be regard as an important factor in the future works. Even more, in order to make the simulated conditions closer to the practical application, stepping from our basic works, some more complex modeling system, including ionic strength and some surfactants will be bring into simulation to discover the more detailed mechanism of interaction between GOx and carbon nanotubes, even other nanomaterials.

ACKNOWLEDGMENT

Feng Liu thanks Prof. Tao Wu and Vice Prof. HaiHua Pan, from the Department of Chemistry, Zhejiang University, for those helpful discussions about some key problems of Molecular Dynamics Simulations.

REFERENCES

[1] Wilson, R.; Turner, A.P.F. Glucose oxidase: an ideal enzyme. Biosens. Bioelectron. 1992, 7, 165-185.

[2] Xiao, Y.; Patolsky, F.; Katz, E.; Hainfeld, J.F.; Willner, I. "Plugging into enzymes": nanowiring ofredoxenzymes by a gold nanoparticle. Science 2003, 299, 1877-1881.

[3] Ramanavicius, A.; Kausaite, A.; Ramanaviciene, A. Biofuel cell based on direct bioelectrocatalysis. Biosens. Bioelectron. 2005, 20, 1962-1967.

[4] Ivnitski, D.; Branch, B.; Atanassov, P.; Apblett, C. Glucose oxidase anode for biofuel cell based on direct electron transfer. Electrochem. Commun. 2006, 8, 1204-1210.

[5] Rivas, G.A.; Rubianes, M.D.; Rodríguez, M.C.; Ferreyra, N.F.; Luque, G.L.; Pedano M.L.; Miscoria, S.A.; Parrado, C. Carbon nanotubes for electrochemical biosensing. Talanta 2007, 74, 291-307.

[6] Davis, F.; Higson, S.P.J. Biofuel cells-Recent advances and applications. Biosens. Bioelectron. 2007, 22, 1224-1235.

[7] Frew, J.E.; Hill, H.A.O. Direct and indirect electron transfer between electrodes and redox proteins. Eur. J. Biochem. 1988, 172, 261-269.

[8] Hess, C.R.; Juda, G.A.; Dooley, D.M.; Amii, R.N.; Hill, M.G.; Winkler, J.R.; Gray, H.B. Gold electrodes wired for coupling with the deeply buried active site of arthrobacter globiformis amine oxidase. J. Am. Chem. Soc. 2003, 125, 7156-7157.

[9] Aguey-Zinsou, K.F.; Bernhardt, P.V.; Kappler, U.; McEwan, A.G. Direct electrochemistry of a bacterial sulfite dehydrogenase. J. Am. Chem. Soc. 2003, 125, 530-535.

[10] Lyons, M.E.G.; Keeley, G.P. The redox behaviour of randomly dispersed single walled carbon nanotubes both in the absence and in the presence of adsorbed glucose oxidase. Sensors 2006, 6, 1791-1826.

[11] Iijima, S. Helical microtubules of graphitic carbon. Nature 1991, 354, 56-58.

[12] Balasubramanian, K.; Burghard, M. Biosensors based on carbon nanotubes. Anal. Bioanal. Chem. 2006, 385, 452-468.

[13] Guiseppi-Elie, A.; Lei, C.; Baughman, R.H. Direct electron transfer of glucose oxidase on carbon nanotubes. Nanotechnology 2002, 13, 559-564.

[14] Cai, C.; Chen, J. Direct electron transfer of glucose oxidase promoted by carbon nanotubes. Anal. Biochem. 2004, 332, 75–83.

[15] Tatke, S.S.; Renugopalakrishnan, V.; Prabhakaran, M. Interfacing biological macromolecules with carbon nanotubes and silicon surfaces: a computer modelling and dynamic simulation study. Nanotechnology 2004, 15, S684-S690.

[16] Shen, J.W.; Wu, T.; Wang, Q.; Kang, Y. Induced stepwise conformational change of human serum albumin on carbon nanotube surfaces. Biomaterials 2008, 29, 3847-3855.

[17] Shen, J.W.; Wu,T.; Wang, Q.; Pan, H.H. Molecular simulation of protein adsorption and desorption on hydroxyapatite surfaces. Biomaterials 2008, 29, 513-532.

[18] Liu, F.; Ye, X. S; Wu, T; Wang, C. T.; Shen, J. W.; Kang, Y. Conformational Mobility of GOx Coenzyme Complex on Single Wall Carbon Nanotubes, Sensors 2008, 8, 8453-8462.

[19] Zhou, H.; Wu, T.; Dong, X.; Wang, Q.; Shen, J.W. Adsorption mechanism of BMP-7 on hydroxyapatite (001) surfaces. Biochem. Biophys. Res. Commun. 2007, 361, 91-96.

[20] Lin, C.S.; Zhang, R.Q.; Niehaus, T.A.; Frauenheim, T. Geometric and electronic structures of carbon nanotubes adsorbed with flavin adenine: a theoretical study. J. Phys. Chem. C 2007, 111, 4069-4073.

[21] Wohlfahrt, G.; Trivic, S.; Zeremski J.; Pericin, D.; Leskovac, V. The chemical mechanism of action of glucose oxidase from Aspergillus niger. Mol. Cell. Biochem. 2004, 160, 69-83.

[22] Michael Meyer, Gerd Wohlfahrt, Jörg Knäblein, Dietmar Schomburg, Aspects of the mechanism of catalysis of glucose oxidase: A docking, molecular mechanics and quantum chemical study, Journal of Computer-Aided Molecular Design, 1998, 12, 425–440.

[23] Hecht, H.J.; Kalisz, H.M.; Hendle, J.; Schmid, R.D.; Schomburg, D. Crystal structure of glucose oxidase from Aspergillus niger refined at 2.3 Å resolution. J. Mol. Biol. 1993, 229, 153–172.

[24] Frey, J.T.; Doren, D.J. TubeGen. University of Delaware: Newark, DE, 2003. Available: http://deaddog.duch.udel.edu/~frey/research/tubegenonline.html.

[25] Patolsky, F.; Weizmann, Y.; Willner, I. Long-range electrical contacting of redox enzymes by SWCNT connectors. Angew. Chem. Int. Ed. 2004, 43, 2113-2117.

[26] Phillips, J.C.; Braun, R.; Wang, W.; Gumbart, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R.D.; Kalé, L.; Schulten, K. Scalable molecular dynamics with NAMD. J. Comput. Chem. 2005, 26, 1781-1802.

[27] MacKerell, A.D.; Bashford, D.; Bellott, M.; Dunbrack, R.L.; Evanseck, J.D.; Field, M.J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F.T.K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D.T.; Prodhom, B.; Reiher, W.E.; Roux, B.; Schlenkrich, M.; Smith, J.C.; Stote, R.; Straub, J.; Watanabe, M.; Wiórkiewicz-Kuczera, J.; Yin, D.; Karplus, M. All-atom empirical potential for molecular modeling and dynamics studies of proteins. J. Phys. Chem. B 1998, 102, 3586 – 3616.

[28] van den Berg, P.A.W.; Feenstra, K.A.; Mark, A.E.; Berendsen, J.C.; Visser, J.W.G. Dynamic conformations of flavin adenine dinucleotide: simulated molecular dynamics of the flavin cofactor related to the time-resolved fluorescence characteristics. J. Phys. Chem. B 2002, 106, 8858-8869.

[29] Hirschfelder, J.O.; Curtiss, C.F.; Brid, R.B. In Molecular theory of gases and liquids; Wiley: New York, NY, USA, 1954.

[30] Humphrey, W.; Dalke, A.; Schulten, K. VMD: visual molecular dynamics. J. Mol. Graphics 1996, 14, 33-38.