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The Molecular Dynamics and Experimental Studies of the Structural Behavior of Alcoholdehydrogenase Enzyme on the Graphitic Sorbent Surfaces

Kholmurodov Kh T[1,2,3,4]*, Baigunov IA[2,3], Gladyshev PP[2], Elhaes H[5] and Ibrahim M[6]

[1]S.U.Umarov Physical-Technical Institute (PhTI), Republic of Tajikistan

[2]Department of Chemistry, New Technologies and Materials, Dubna State University, Russia

[3]Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Russia

[4]Department of Fundamental Nuclear Interactions, Faculty of Physics, Lomonosov Moscow State University, Russia

[5]Physics Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Egypt

[6]Molecular Spectroscopy and Modeling Unit, Spectroscopy Department, National Research Centre, Egypt

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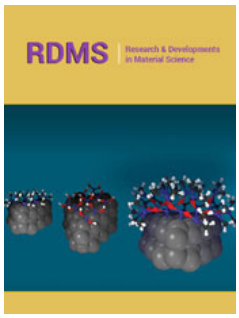

***Corresponding author:** Kholmurodov Kh T, Department of Chemistry, New Technologies and Materials, Dubna State University, 141980, Dubna, Moscow Region, Russia

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Abstract

In the present work the computer molecular dynamics and experimental studies have been performed for the enzyme alcoholdehydrogenase with its co-factor (ADH+NAD) solvated by water on a graphitic carbon surface. The issues of orientation of protein sorption on matrices of various sorbents are also covered. The numerical MD modeling implemented in this study use the AMBER-18 package with a fast module realization "pmemd.cuda" on a CPU/GPU cluster machine. The MD analysis provides mapping of the orientation adsorption of the ADH+NAD enzyme with a significant extension of the original basic model, thereby allowing the change in protein conformation observed in detail in the region of the ADH titratable amino acid residues. The detection of the characteristic conformation of key titratable amino acids may become a necessary stage in further research and implementation of a numerical experiment, which will be carried out by varying the pH and charge values. Next, based on the extension of the molecular dynamics (MD) model implementation the mechanism of conformational changes in the whole system (ADH+NAD + water / graphitic carbon surface) is examined and the orientation aspects of the whole protein system along with the key titratable amino acids are studied in detail. The MD simulation data discussed with the experimental observations, which indicate on the atomic/molecular mechanism of the influence of pH solution on the proteins' conformation and orientation adsorption.

Keywords: Proteins' conformation; Water solution; Graphitic carbon surface; Alcohol dehydrogenase enzyme; Molecular dynamics; Experimental observations; Adsorption processes

Introduction

Computational methods offer important ways to describe and understand the atomic/molecular phenomena in many physical and bio-chemical systems. These methods are widely used in physics, chemistry as well as biological sciences, in computer design of new materials and drugs [1-5]. In this work, computer Molecular Dynamics (MD) modeling and experimental studies have been performed for the alcoholdehydrogenase (ADH) enzyme with its co-factor Nicotinamide Adenine Dinucleotide (NAD) in an aqueous solution interacting with a graphitic carbon surface. The numerical experiment implemented in the study facilitates the AMBER-18 package ("pmemd.cuda") and aim on a mapping of the ADH+NAD enzyme orientation adsorption. The MD analysis data provides a significant extension of

the original basic model, thereby allowing the change in protein conformation in the region of the titratable amino acid residues of ADH in atomic/molecular detail. Detection of the characteristic conformation of key titratable amino acids may become a necessary stage in further research and implementation of a numerical experiment, which will be carried out by varying the pH values and surface charge [6-12]. It's worth noting that in modern life science the issues of studying protein-protein interactions, interactions of enzymes with coenzymes and substrates, as well as the sorption of proteins, taking into account various chemical interactions with solvent molecules and specific fragments of the surface of sorbents, are the most intriguing problems due to the sufficient important applications in nanobiotechnology, biochemistry, etc. These interactions are determined by hydrogen, hydrophobic, ionic, electrostatic interactions, as well as their combinations. The pH of the solution has a particularly strong influence on the conformation and interactions of proteins, which determines the ionization state of the ionogenic groups of proteins [6-8].

The Experimental Measurement Data

In a series of the experiments performed by Prof. P.P. Gladyshev and co-authors [13-15] the aspects of the ADH orientation (Figure 1 & 2) on various sorbents and electrically conductive matrices were investigated using the experimental observations of the protein depending on the solution pH. It should be noted that experimental study of these issues outlined above is difficult. Therefore, in recent years, computational and simulation analysis methods have been widely used for these purposes.

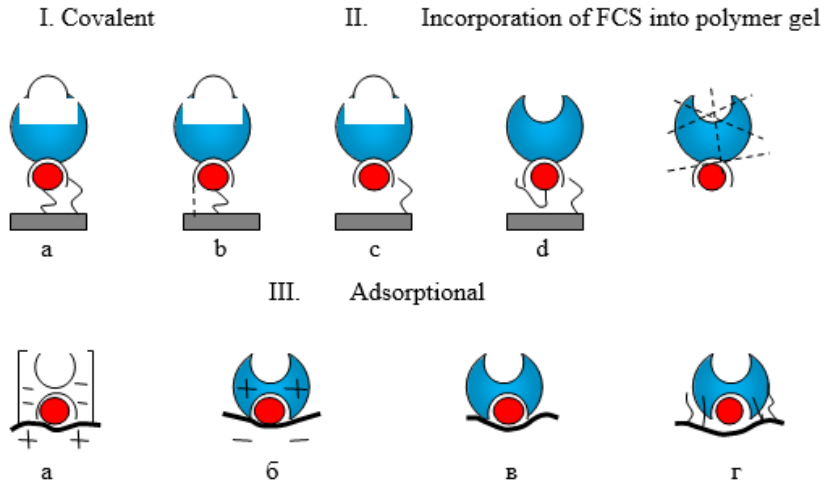


Figure 1: Methods for immobilization of enzyme-cofactor systems on electrically conductive carriers [13-15].

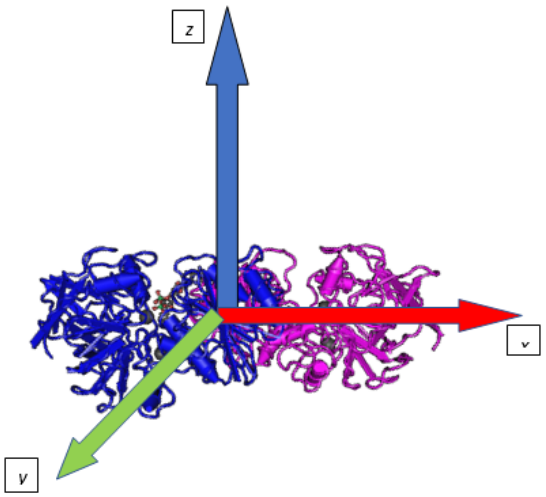


Figure 2: A crystal structure of the alcohol dehydrogenase (ADH) complex is shown. Blue, red and green arrows are introduced with the subsequent purpose of studying the molecular dynamics processes of interaction of the ADH molecule with the surface of the sorbent, determining the most probable orientation direction and adsorption site of ADH on the surface (see below, for example, Figure 5). The arrows are directed from the center of the ADH to the region containing titratable amino acids, sensitive to changes in the pH values of the solution, towards the catalytic gaps and substrate centers [13-15].

At the same time, effective methods of Molecular Dynamics (MD) and molecular modeling represent one of the most powerful approaches [3-5,16-18]. In relation to the problems of modeling the effect of pH on the orientation of protein sorption, the sorption behavior of the alcohol dehydrogenase enzyme was previously studied in [6-15], based on the specific interactions of protein groups with groups on the surface and its charge. It was assumed that the conformation of the alcohol dehydrogenase enzyme corresponds to its conformation in the crystal structure and does not change when the surrounding solution and sorbent surface change, which is a fairly rough approximation. The implementation of various options includes, for example, the immobilization of two-substrate enzyme on the surface of electrode materials (Figure 1).

The experimental and literature data have been analyzed with regards to the proteins' adsorption on various solid surfaces, such as glass, quartz, kaolin, aluminum oxide, cellulose, various inorganic powders and polymer membranes. The sorption behavior of proteins depends on many factors. The proteins and enzyme sorption on ion-exchange sorbents depends on the degree of ionization of the ionogenic groups of the sorbent and protein, which is determined by the properties of the functional groups and the medium. Sorption is significantly influenced by the nature of the electrolyte, the structure of the sorbent, and its ability to swell. The stronger the ionic binding of the enzyme to the oppositely charged matrix, the greater the charge of the protein, determined by the pH in the ion exchanger phase. In ion exchange binding, in general, the pH of the optimum enzyme activity may not coincide with the pH at which the strongest binding of the enzyme occurs. The adsorption of charged products formed as a result of the reaction on the carrier also negatively affects the activity of the enzyme. Thus, it can be stated that the activity of the immobilized system is determined by the totality of the properties of the enzyme, the carrier, as well as the conditions under which the enzymatic process is carried out. The disadvantage of pure electrostatic immobilization is the instability of the resulting systems in solutions with high ionic strength and at a pH environment in which the protein globule and the carrier have like charges [6-15].

Below for the AHD (alcoholdehydrogenase) the dependence of the degree of protonation on the pH of the solution is shown. During the calculations we have generated 36 files at different pH values and a graph of the degree of protonation as a function of pH was thus plotted (Figure 3).

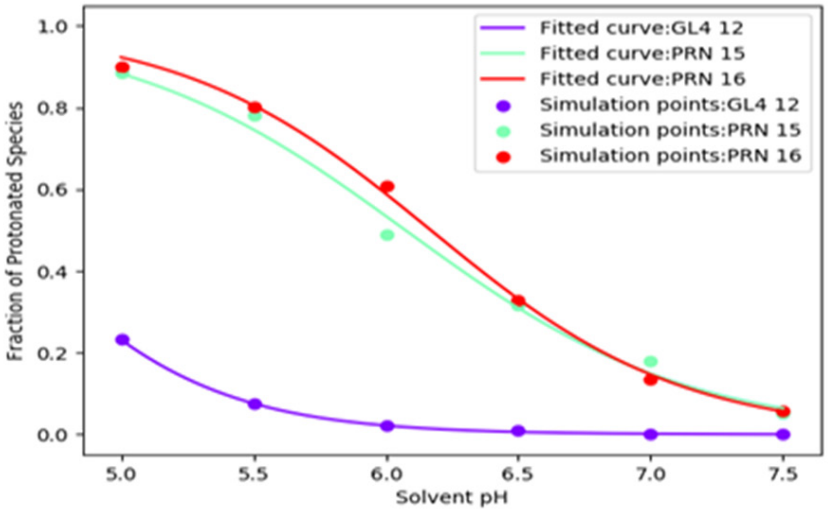


Figure 3: The dependence of the degree of protonation on the pH of the solution.

The interactions energies between the protein side groups and surface of the sorbents were set equal to the energies of their interactions found from chromatographic data (Figure 4); [13-15].

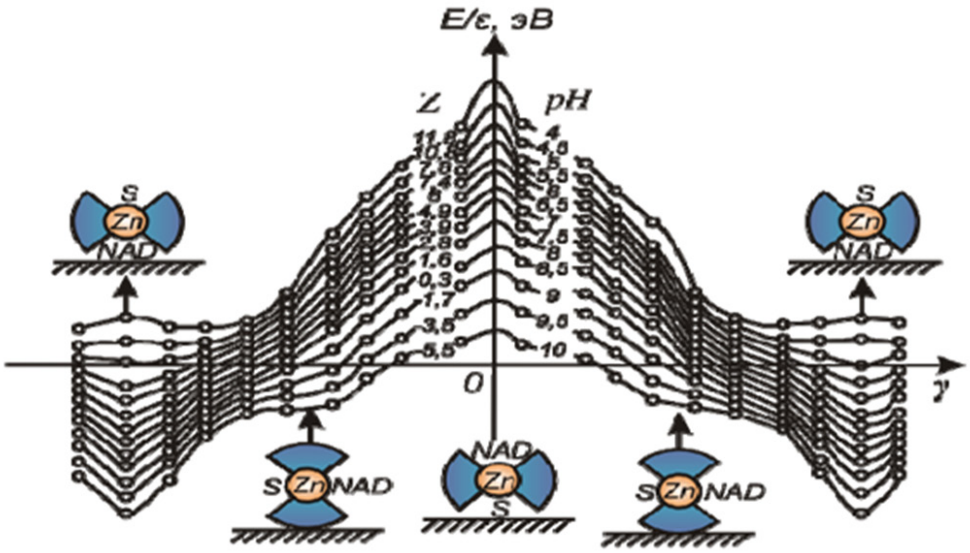


Figure 4: The dependence of the energy of electrostatic interaction of ADH (alcohol dehydrogenase) with a positively charged surface from the angle of rotation of the globule around its axis, passing through the center of mass along the maximum size of the globule, and pH of the solution (Z is the total charge of the globule) [13-15].

Such model made it possible to identify a clear orientation of the sorption of this protein on the surface of ion-exchange and reverse-phase sorbents. Later, one of the co-authors of this article carried out work [13-15] on oriented sorption. Table 1 shows the pKa values for alcohol dehydrogenase amino acids used in the modeling.

Table 1: pKa values for alcohol dehydrogenase amino acids used in sorption modeling [13-15].

Aminoacids	Amount	Range of pKa Variation in Globular Proteins	pKa Used in the Calculation for Alcohol Dehydrogenase
α-NH_2 (end)	2	7,60-8,40	8,35
Arg	24	11,60-12,60	12,60
His	14	5,60-7,00	7,00
Lys	60	9,40-10,60	10,60
α-COOH (end)	2	2,10-3,20	2,54
Tyr	8	9,40-10,50	10,50
Glu	42	4,20-4,60	4,60
Asp	34	3,00-4,70	3,58
Cys	28	8,30-9,20	9,10

The Molecular Dynamics Method

In this section, we present descriptions of the main parameters and algorithms used in the computer Molecular Dynamics (MD) simulations. In this study, we have used both CPUs and GPUs - based computing environment to perform MD simulation with AMBER. We used several computing environments such as cluster with 16 cores, GPU Geforce (GPU=(GTX 1080Ti)) to realize the running MD simulations in Amber18 (pmemd) with GPU acceleration (pmemd. cuda). We refer on the program code and the Amber 2018 Reference Manual [3,16-18].

The calculations were carried out on servers at the Heterogeneous platform "HybriLIT" of the Multifunctional Information and Computing Complex (MICC) of the MLIT (Meshcheryakov Laboratory of Information Technologies) of JINR (Joint Institute for Nuclear Research). Heterogeneous platform consists of "Govorun" supercomputer and "HybriLIT" education and testing polygon at the JINR, Dubna, which are as a two-component system that includes CPU-component based on the newest Intel architectures (Intel Xeon Phi and Intel Skylake processors), and GPU-component based on NVIDIA DGX-1 Volta. Some calculations were performed on a local server at the FLNP (Frank Laboratory of Neutron Physics) of the JINR using two 4-core 64-bit Intel Xeon E5-2640 processors with a clock frequency of 2.4 GHz and 8 GB of RAM, running on the Linux CentOS version 8 operating system. The local server includes NVIDIA Corporation GP104 [GeForce GTX 1070] with Intel Xeon E7 v4/Xeon E5 v4/Xeon E3 v4/Xeon.

We have realized the main production MD (CPU/GPU) simulations (common also with many other types of simulations) for the PDB ID: 3COS of the crystal structure of human class II alcohol dehydrogenase (ADH); PDB DOI: https://doi.org/10.2210/pdb3COS/pdb.

The model building, the AMBER/GPU performance with pressure control peculiarities

We have employed the Molecular Dynamics (MD) simulations with Amber 18 code (CPU/GPU medium). The MD modeling on the molecular system ADH+NAD + water + carbon surface (below Figure 5a-5c) was carried out in three stages: the energy minimization, NVT and NPT relaxation procedures. The preparation stages (energy minimization, NVT and NPT equilibration) have been fulfilled out for 10 ns dynamical relaxation changes.

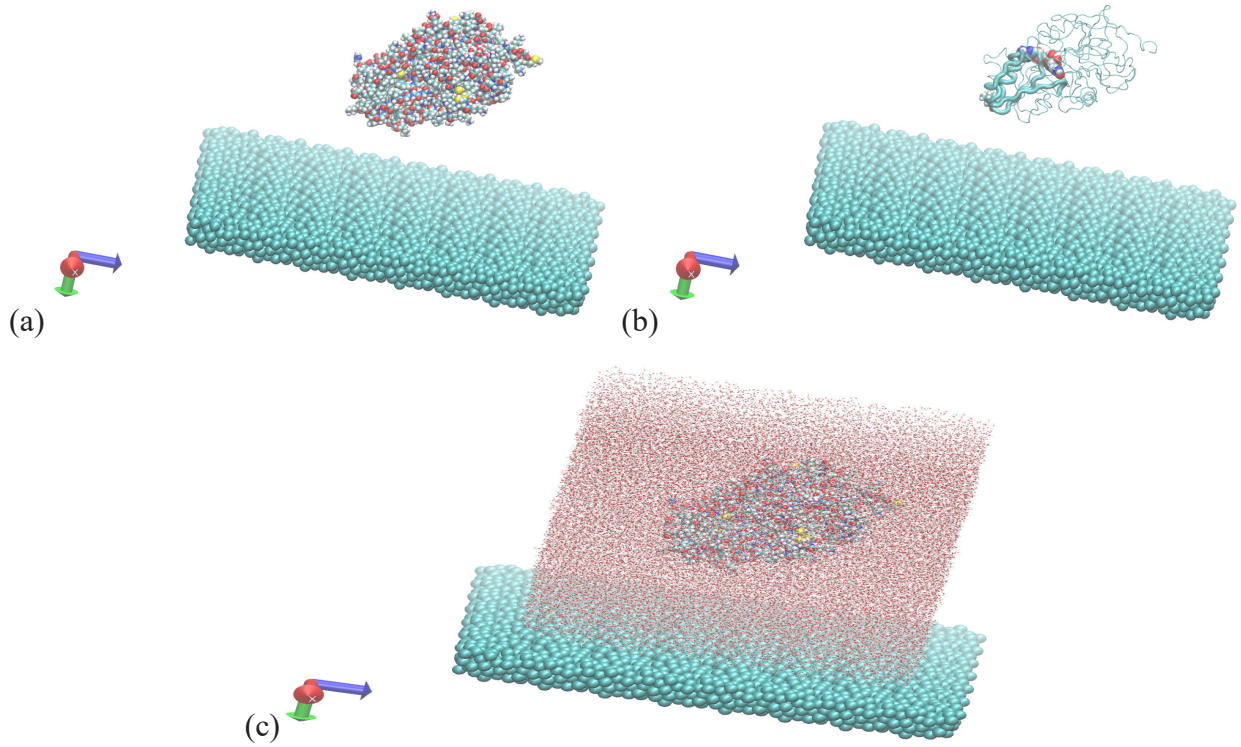


Figure 5(a-c): A general molecular design of the system (ADH+NAD + water + graphitic carbon surface).

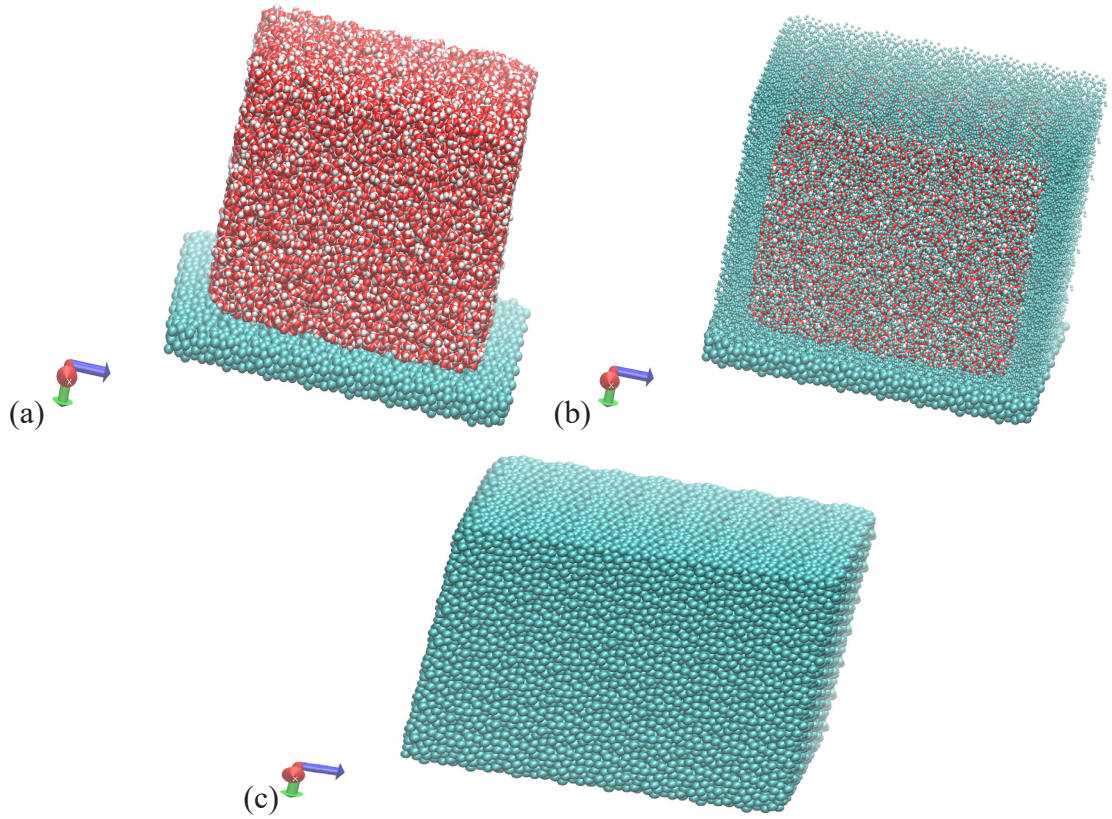


Figure 6(a-c): A general molecular design for the system ADH+NAD + water + graphitic carbon surface.

It's worth noting that the Amber' GPU code used and due to the performance reasons the GPU code does not recalculate the non-bond list cells with an error related to skinnb. Once the system is equilibrated the box size fluctuations should be small and so this should not be an issue during production. We have replicated the graphitic carbon surface as six periodic plates, thereby symmetrically surrounding the ADH+NAD + water system in all the spatial directions due to the pressure control and periodic boundary conditions. Thus, the whole system, the enzyme ADH+NAD solvated by TIP3P water, was generally modelled as a very big cubic system consisting of the thermostating and confining graphitic carbon surfaces (Figure 6a-6c).

The MD production with SHAKE on for bonds with hydrogen, no force evaluation for bonds with hydrogen and Langevin thermostat has been carried out at temperatures T = 303K. The constant pressure periodic boundary conditions and anisotropic pressure coupling and nonbonded cutoff 10 Å were used. The MD simulations in Amber18 with CPU/GPU (pmemd/pmemd.cuda) accelerations have done for the three general setup phases that contain the main production MD:

1) Minimize system to relax bad contacts.
2) Heat the system slowly to the target temperature.
3) Equilibrate the system at the target temperature.

In the first stage, we minimized the system applying restraint on backbone atoms and on some atoms. The initial charge distribution on the titratable residues corresponds to the default states on leaprc.constph and leaprc.conste in tleap. The minimization was performed using sander instead of pmemd to warrant and evaluate the energies behave during the minimization stage. Next, we performed a heating simulation at constant volume on the minimized structures to heat the system slowly from 10 to 300K during some initial steps 2.0ns of simulation with a target temperature of 300K. We keep the restraints on the backbone atoms, but with a weaker force constant in comparison to the one used during minimization. These simulations performed using pmemd, pmemd.MPI, sander or sander.MPI instead of using the GPU-accelerated code with pmemd.cuda. In the equilibration stage, we equilibrate the system (AHD+NAD enzyme + water) at the target temperature of 300K. The explicit solvent simulation is performed at constant pressure to allow the system density to be stabilized. The simulations are performed for 20ns in implicit and explicit solvent. Below the Table 2 presents the input file for the simulations using explicit solvent model.

Table 2: The equilibration stage for ADH alcohol dehydrogenase in explicit solvent [1].

model.explicit.mdin
The Equilibration Stage in Explicit Solvent
&cntrl
imin=0, ! Molecular dynamics and Equilibration (=1, Minimization)
ntc=2, ! SHAKE constraints (=2, hydrogen bond lengths constrain.)
ntf=2, ! Force evaluation (=2, hydrogen bond interact.s omitted)
ntb=1, ! Boundaries (=1, constant volume)
cut=9.0, ! Cutoff
dt=0.002, ! The time step in picoseconds
nstlim=10000000, ! Number of MD steps performed
ig=-1, ! Random seed (=-1, get a number from date and time)
ntwr=10000, ! Restart file written every ntwr steps
ntwx=10000, ! Trajectory file written every ntwx steps
ntpr=10000, ! The mdout and mdinfo files written every ntpr steps
ioutfm=1, ! Trajectory file format (=1, Binary NetCDF)
iwrap=1, ! Translate water molecules into original simulation box
igb=0, ! GB model (=0, explicit solvent)
saltcon=0.1, ! Salt concentration
irest=1, ! Flag to restart the simulation
ntx=5, ! Initial cond. (=5, coord. and veloc. read from inpcrd)
&end
&ewald
chngmask=0, ! Only required to avoid SANDER error
ew_type=0,
skinnb=1.0,
&end

Results and Discussions

The ADH energy evaluation results

The MD calculation results for ADH (alcoholdehydrogenase) have shown in Tables 3 & 4. The data were gathered for the different type of the energies to compare the dependence on the pH.

Table 3: The MD calculation results for ADH energies at different pH values.

The pH value and energy	pH=6	pH=7	pH=8
E_{tot}, , kJ/mol	-4140	-3700	-4720
E_{elec}, kJ/mol	-111242	-110344	-110912
E_{ptot}, kJ/mol	-22075	-22075	-22672
E_{VDW}, kJ/mol	-10437	-10726	-10655

Table 4: The MD calculation results for ADH chemical bond energies at different pH values.

The pH value and energy	pH=6	pH=7	pH=8
E_{bond}, kJ/mol	4820	5008	4761
E_{angle}, kJ/mol	13171	13414	13614
E_{dihed}, kJ/mol	16390	16335	16172

It is important to evaluate the relationship of the MD data shown in Tables 3 & 4 with conformational changes of the ADH (alcoholdehydrogenase) molecule in dependence of the pH value.

Mapping the orientation of titratable aminoacid residues for the ADH enzyme

This section, subsequently, presents the numerical experiment on the orientational peculiarities of the ADH (alcohol dehydrogenase) enzyme with using the AMBER (pmemd.cuda) package calculations die to the expansion of the positional changes of its key titratable aminoacid residues. The previously mentioned structure of the ADH molecule (a human class II alcohol dehydrogenase; DOI: https://doi.org/10.2210/pdb3COS/pdb; PDB ID: 3COS) contains 379 aminoacid residues starting from GLY1 and ending PHE379. From the ADH titratable aminoacid residues (from Table 1 above) we have selected the following titratable amino acid residues: ASP54, HIS252 and ASP268. The MD simulation results in Figure 7a-7c show their orientation positions. The comparison of the ADH orientation with titratable aminoacid residues ASP54, HIS252, ASP268 at the (a) initial state of the ADH molecule (t=0), (b) t=10 ns and (c) t=20 ns relaxed states.

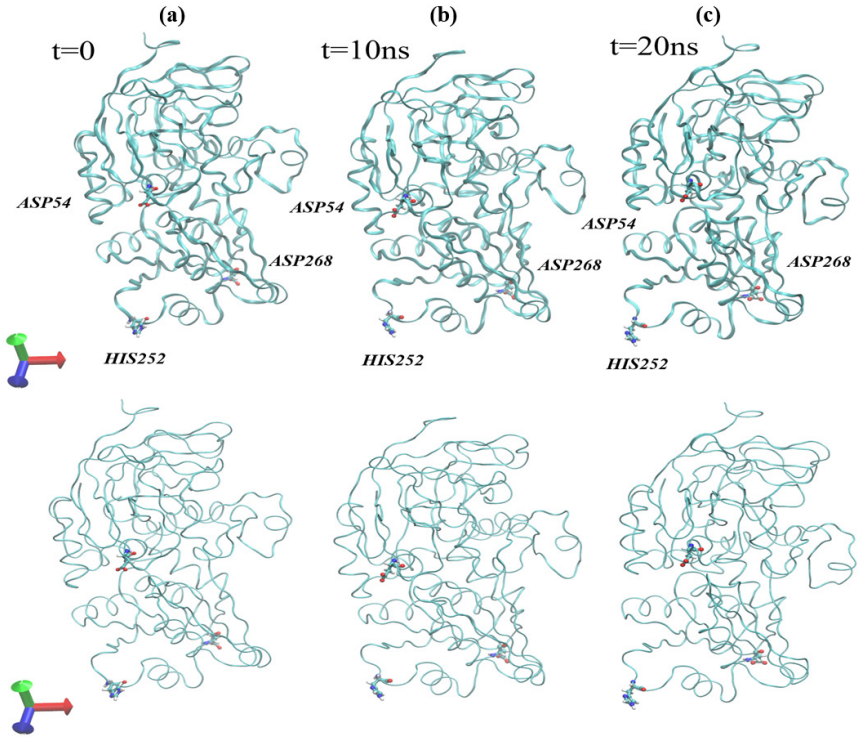


Figure 7(a-c): The comparison of the ADH orientation with titratable aminoacid residues ASP54, HIS252, ASP268 at the (a) initial state of the ADH molecule (t=0), (b) t=10 ns and (c) t=20 ns relaxed states.

Below in Figure 8a-8c we have presented the side and top position views of each from these three titratable amino acids (ASP54, HIS252 AND ASP268), thereby illustrating the orientation mapping detail.

From Figure 8a-8c that we must observe a stable relaxed conformation of each titratable aminoacid residues as results of the multi-million dynamical conformational changes of the ADH molecule surrounded by the water solvent. It is noteworthy that the orientation of HIS252 has to possess a visible change among the three titratable amino acid residues ASP54, HIS252, ASP268. The orientation detail of the titratable aminoacid residue HIS252 have shown in Figure 9 (side and top views) at t=0, t=10ns and t=20ns relaxed states. The comparison of the orientation of the ADH titratable aminoacid residues are strait forward (below Figure 9 & Table 5).

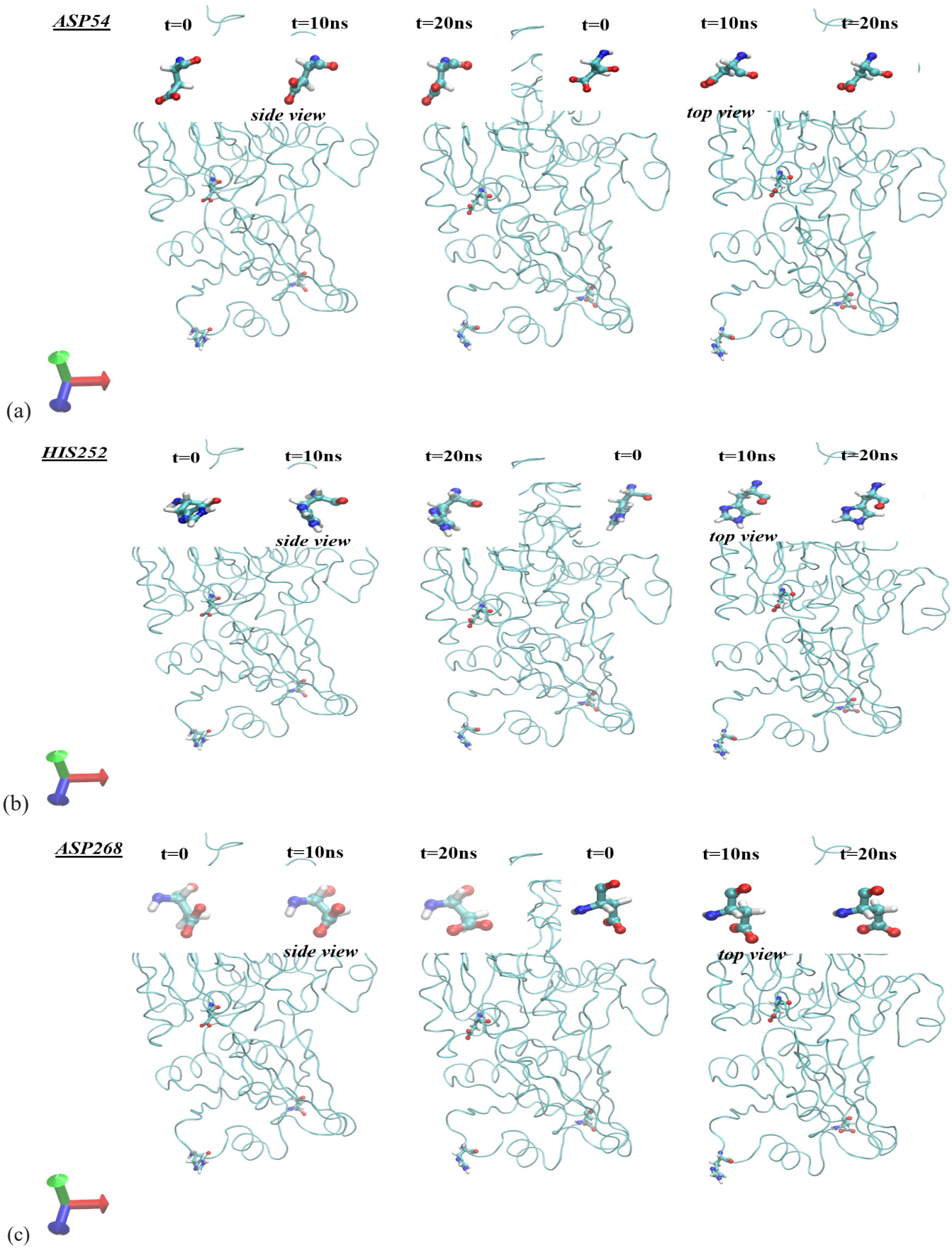


Figure 8(a-c): The comparison of the ADH orientation with titratable aminoacid residues ASP54, HIS252, ASP268 (the side and top views are shown at t=0, t=10 ns and t=20 ns relaxed states).

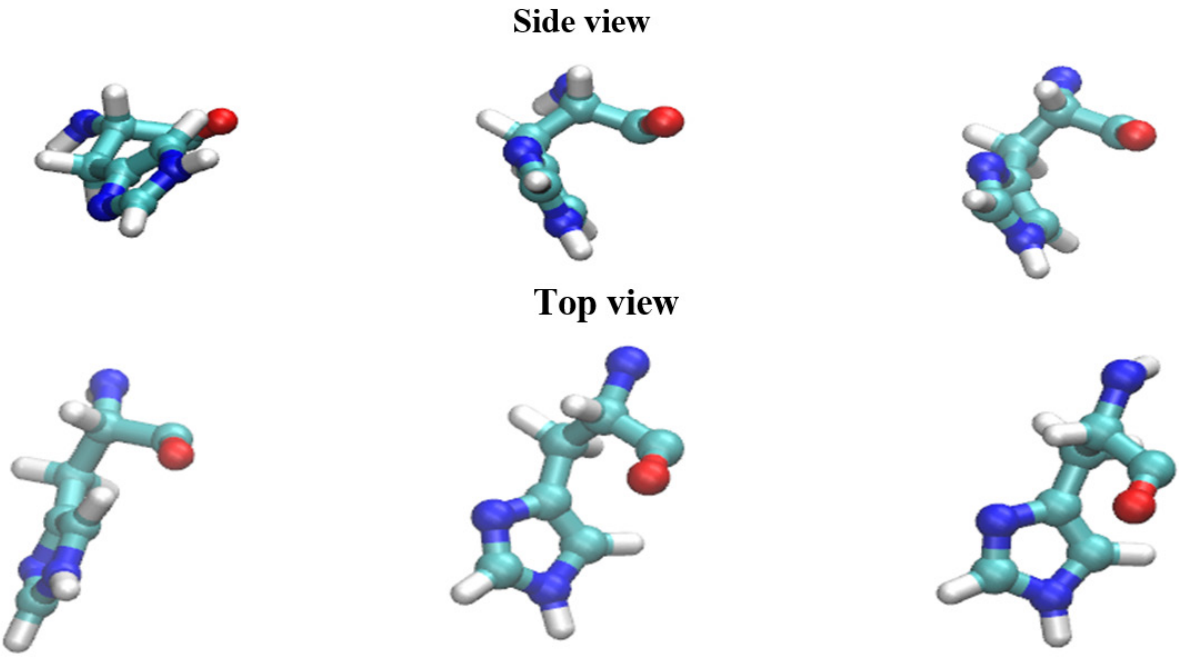


Figure 9: The orientation detail of the titratable aminoacid residue HIS252 have shown at t=0, t=10 and t=20 ns relaxed states (the side and top views are shown from left to right).

Table 5: The MD calculation results for the ADH titratable aminoacid residues on the dependence of the rotation angles with time.

	Ψ(t = 0 нс)	Ψ(t = 10 нс)	Ψ(t = 20 нс)
ASP-54	64.5°	58°	66.3°
HIS-252	46.1°	-60.7°	57.1°
ASP-268	-164.7°	-171.8°	-176.7°

The ADH+NAD adsorption dynamics on the graphitic carbon surface

Now we consider the ADH+NAD adsorption dynamics behavior on the graphitic carbon surface. Below the MD/AMBER calculations results were done using the Visual Molecular Dynamics (VMD) program (Figure 10a-10c). The snapshots of the Figure 7a-7c present the MD calculation results with (ntb=1) periodic boundaries (PME) constant volume when minimizing and initially heating / equilibrating the total system, ADH+NAD + water solvent + graphitic carbon surface, and with (ntb=2) periodic boundaries (PME) constant pressure while using for a production run once, we have heated and equilibrated at constant volume. It's worth noting that finding the ADH+NAD + water + surface relaxed equilibrium states have to be a slow process, so far for each set of the MD models we have fulfilled 100 ns calculations using the extremely fast module "pmemd.cuda". Thus, we have completed several MD run for each around fifty-million-time steps (nstlim=50000000, dt=0.002, cut=9.0, ntt=1) at the room temperature (temp0=300). To keep the surface atom positions fixed we have used the restraint options (ntr=1, restraint_wt=25, restraint mask=':383-35140'), so far the positional restraint was 25 kcal/mol*Ang^-2 for this set of the MD calculations. It's worth noting that the ntr=1 means that we have turned on position restraints and therefore have to specify via GROUP input the atoms (graphitic carbon surfaces) were restrained as well as the force constant. In this run, after specification of the namelists, a title is given, followed by the force constant for the restraint (in kcal mol-1 angstrom-2) and then a specification of surface carbon (C) residues or atoms to restrain. Residues can be specified using the «RES» keyword. We have chosen a force constant of 25 kcal mol-1 angstrom-2 and restrained the surrounding ADH+NAD + water confined C-surfaces residues were through 383-35140 (from 1 to 382 the correspondent numerations stand for the ADH+NAD enzyme; a total number of atoms for the ADH+NAD + water + carbon surfaces were 157371).

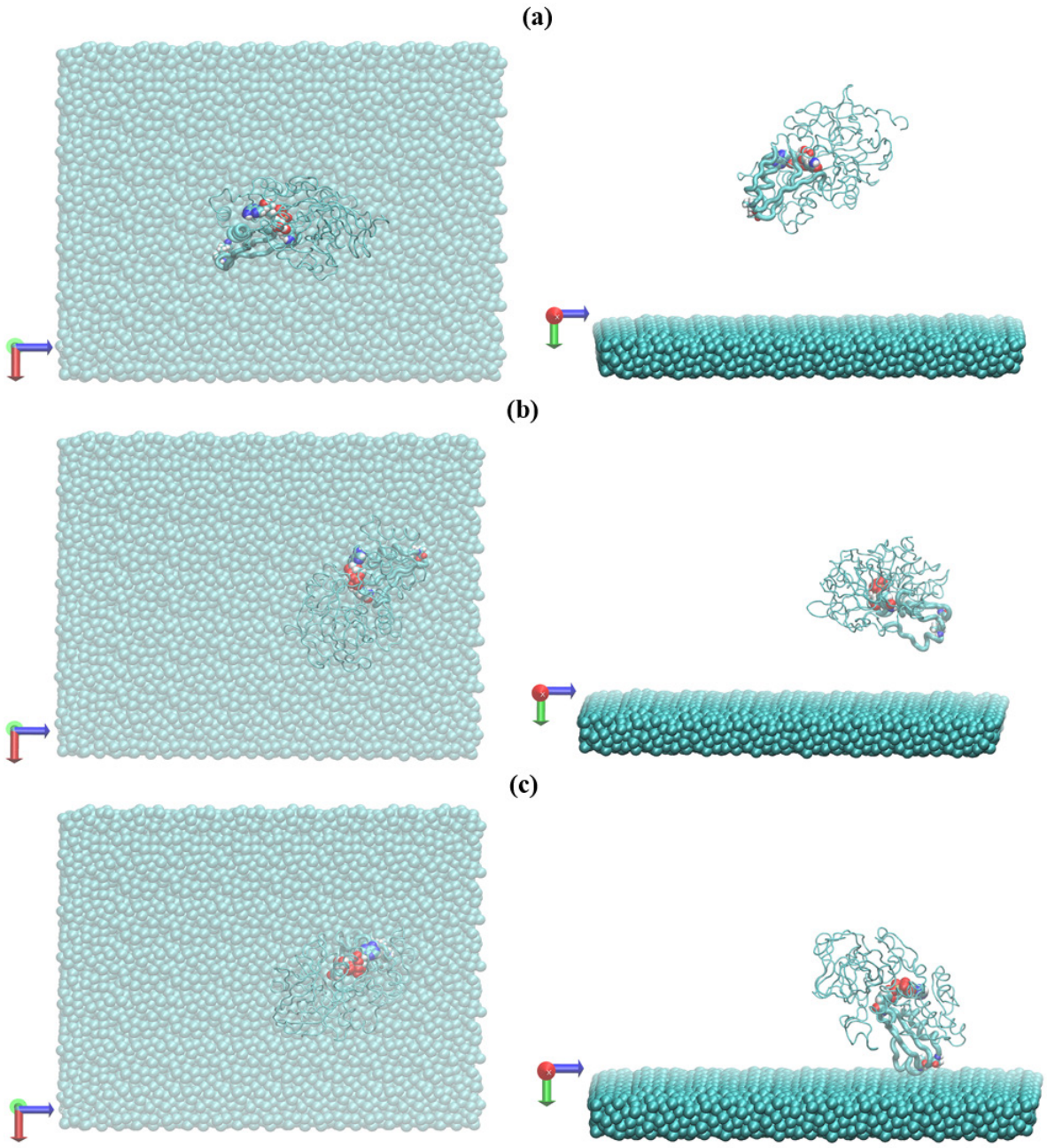
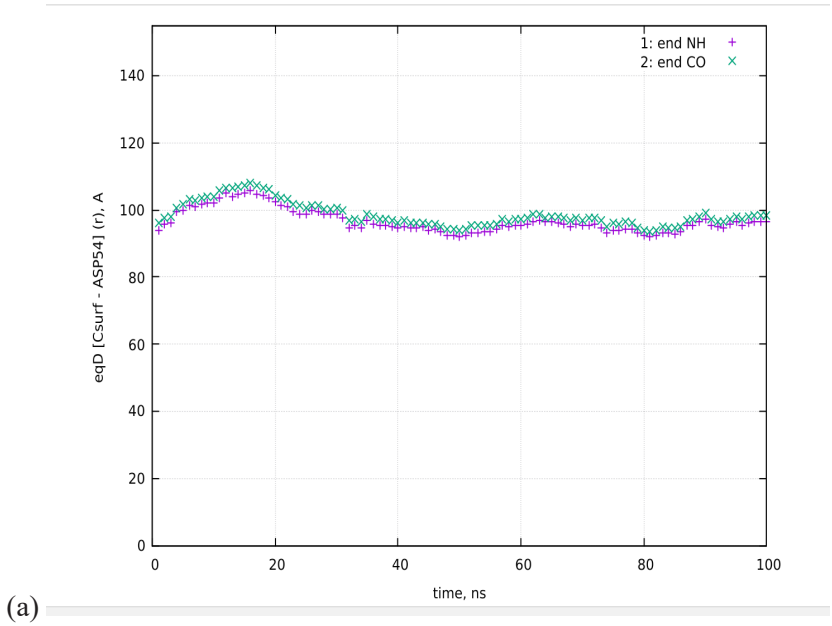


Figure 10(a-c): The ADH+NAD adsorption on the graphitic carbon (C-surface) for the 100 ns dynamical and conformational changes.

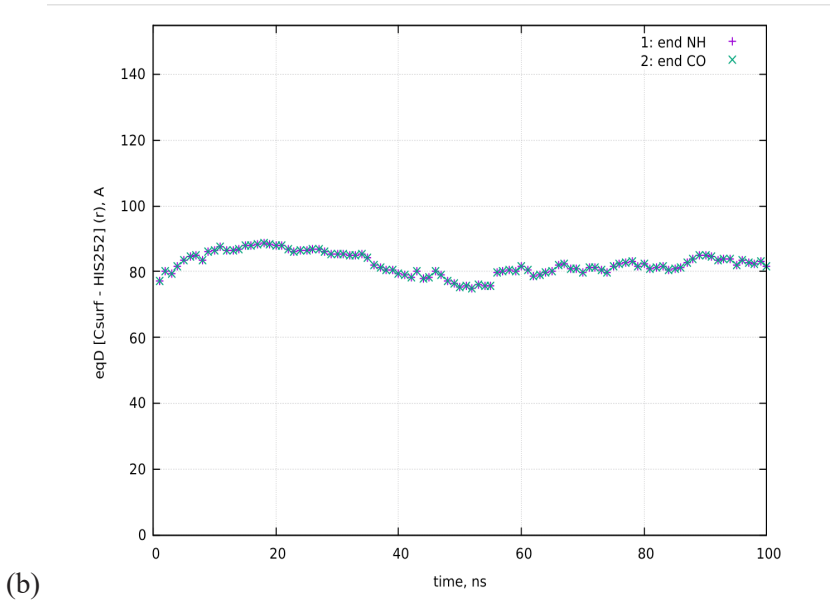
In Figure 10a-10c we present the resulting pictures of the ADH+NAD adsorption process on the graphitic carbon surface during the long 100 ns dynamical changes: (a) - initially relaxed state; (b) - intermediate and (c) - final equilibrium states. The ADH+NAD enzyme before adsorbing on the given graphitic carbon (C-surface) has gone through multi-millions conformational and rotational changes to be finally captured and relaxed on the surface.

The ADH+NAD structural conformations and titratable aminoacid residues

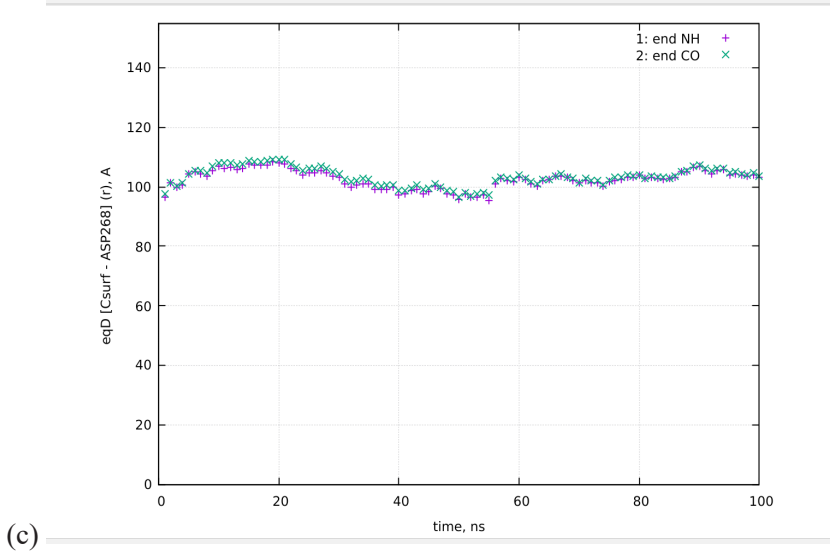
In this section we present the MD calculation results for the ADH+NAD + water / C-surface to display the ADH+NAD structural conformations through the behavior of the above three titratable amino acid residues - ASP54, HIS252 and ASP268 at the initial and final states. Figure 11a-11c display the initially relaxed state during the ADH+NAD adsorption process to be established above the graphitic carbon surface (C-surface). It's worth noting that we have selected one C-atom (Figure 12a-12b) from the confining graphitic surface in the vicinity of the ADH+NAD adsorbing site to. The snapshots in Figure 12 show the position of the selected C-atom for the (a) - an initially equilibrium state and (b) - a final state after 100 ns interaction dynamics ADH+NAD + water / C-surface. Next, in Figure 13a-13c we present the distance calculations of the same titratable amino acid residues ASP54, HIS252 and ASP268 from a selected atom on the C-surface at final state after the long 100ns dynamical changes.



(a)



(b)



(c)

Figure 11(a-c): The MD calculation results for the distance dependence of the ADH+NAD/C-surface of the above three titratable amino acid residues - ASP54, HIS252 and ASP268 at the initially relaxed state.

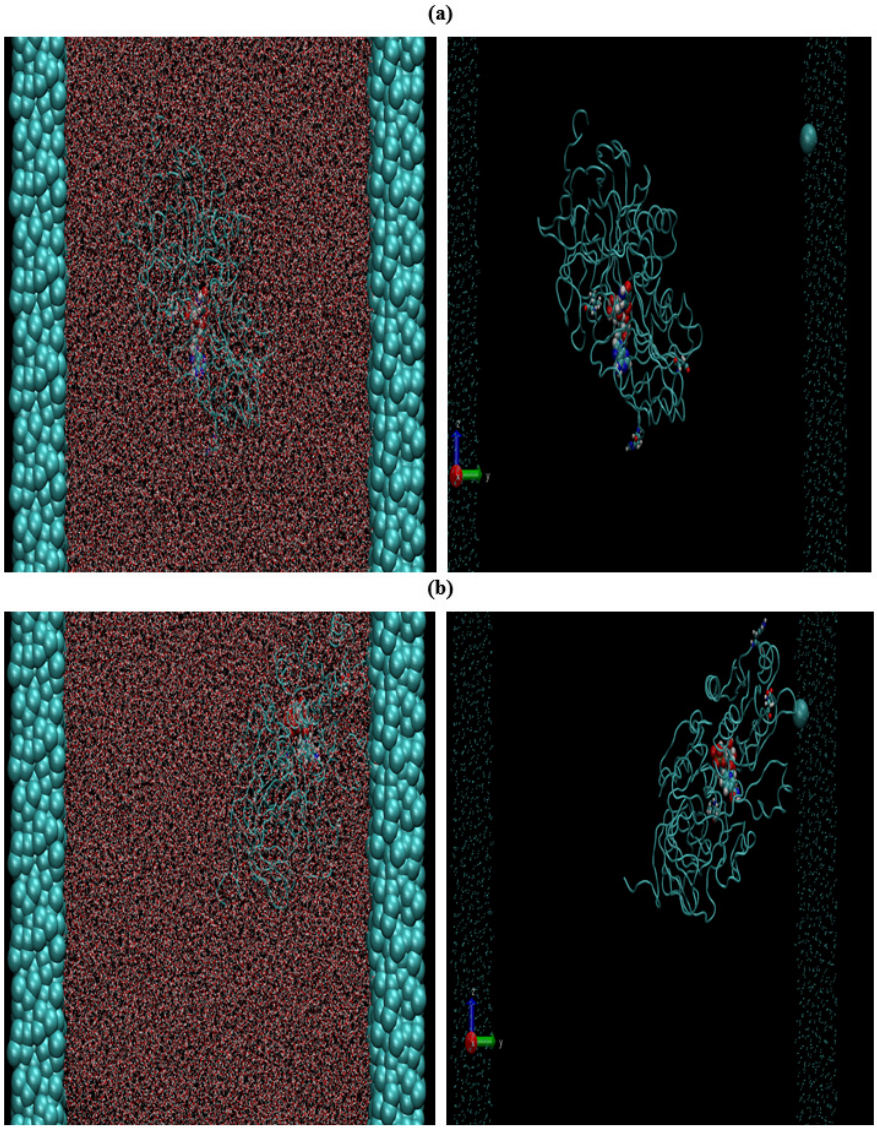
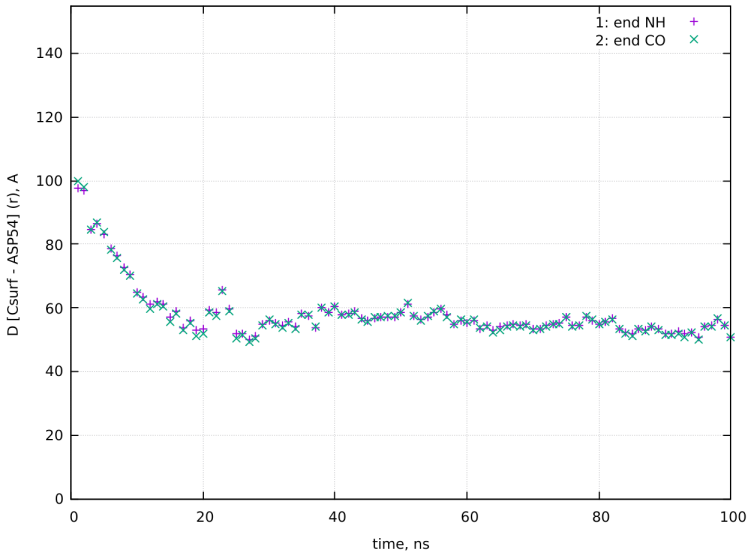
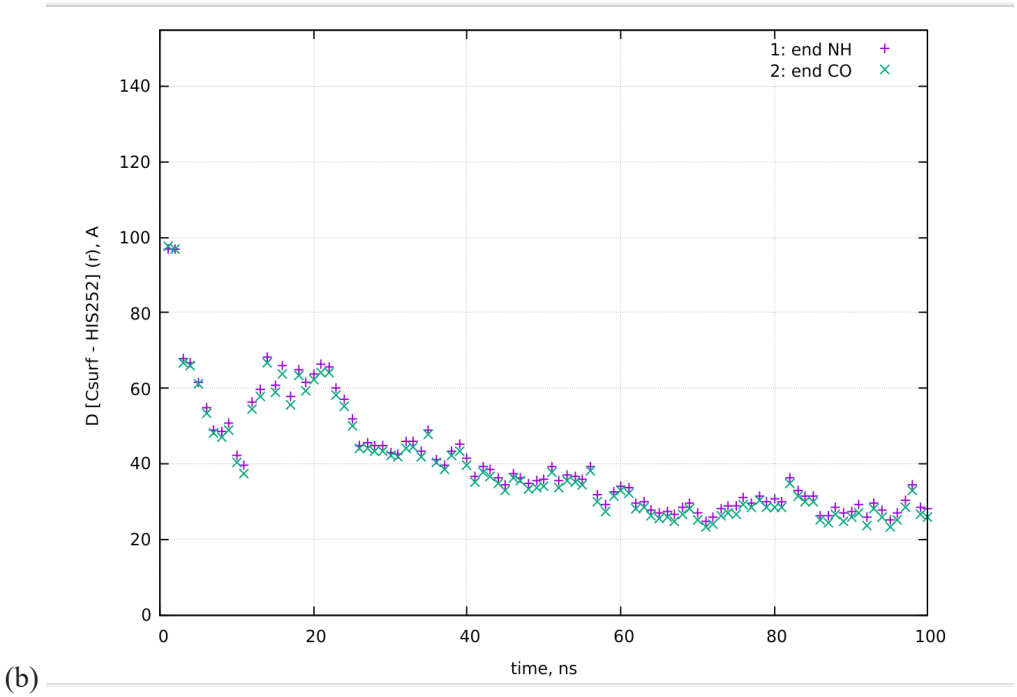


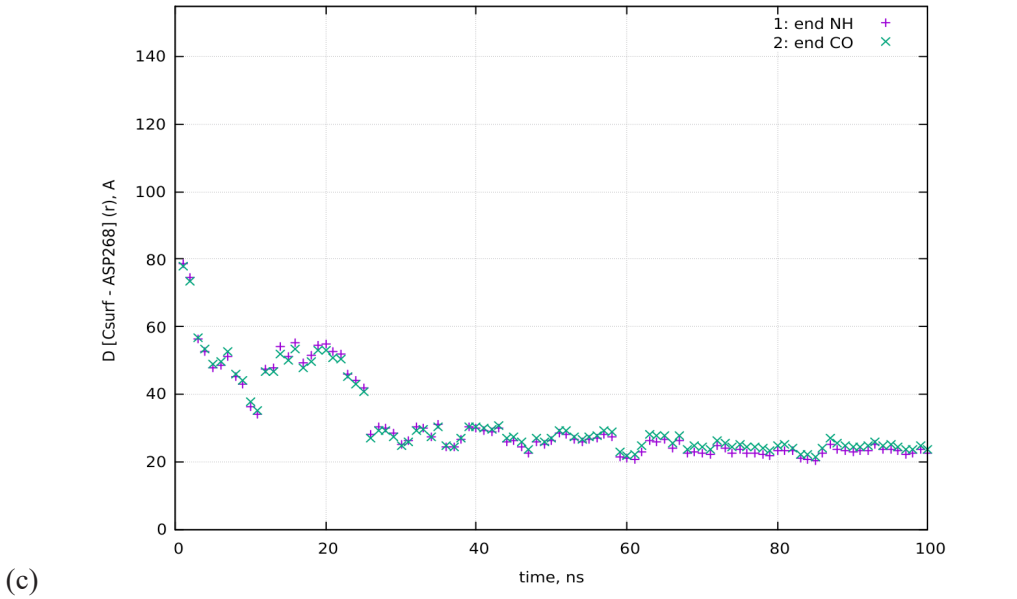
Figure 12 (a-b): The initial and resulting positions of the ADH+NAD + water on the C-surface during 100 ns dynamical and orientation changes. The ADH+NAD positions on the C-surface have shown for (a) - an initially relaxed state and (b) a final adsorbing position of the molecule at a final state of t=100 ns.



(a)



(b)



(c)

Figure 13 (a-c): The MD calculation results for the distance dependence of the ADH+NAD/C-surface of the above three titratable amino acid residues - ASP54, HIS252 and ASP268 at the final state (t=100 ns).

The ADH+NAD structural conformations and behavior of the enzyme catalytic loops

One of the nontrivial events in the conformational structural dynamics of the whole ADH+NAD + water / C-surface system and tracing the individual amino acid residues must be behavior of the enzyme catalytic loops. Below Figure14a-14c shows the ADH+NAD / C-surface dynamical pictures and adsorption processes accompanied with the gradual changes in the orientation of the two catalytic loops of the ADH+NAD molecule relative to a given C-atom on the graphitic surface. The important observation is that these two catalytic loops are closely positioned inside the ADH+NAD molecule, while reaching the adsorbing graphitic surface we can see the separating these loops from each other. A key summary of the entire process, as shown in Figure 14a-14c from the side and top views, has to be separating from each other hence opening the ADH+NAD important catalytic loops) as a result of the influence of the adsorbing C-surface (in Figure 14a-14c these two enzyme loops are shown as solid lines fragments).

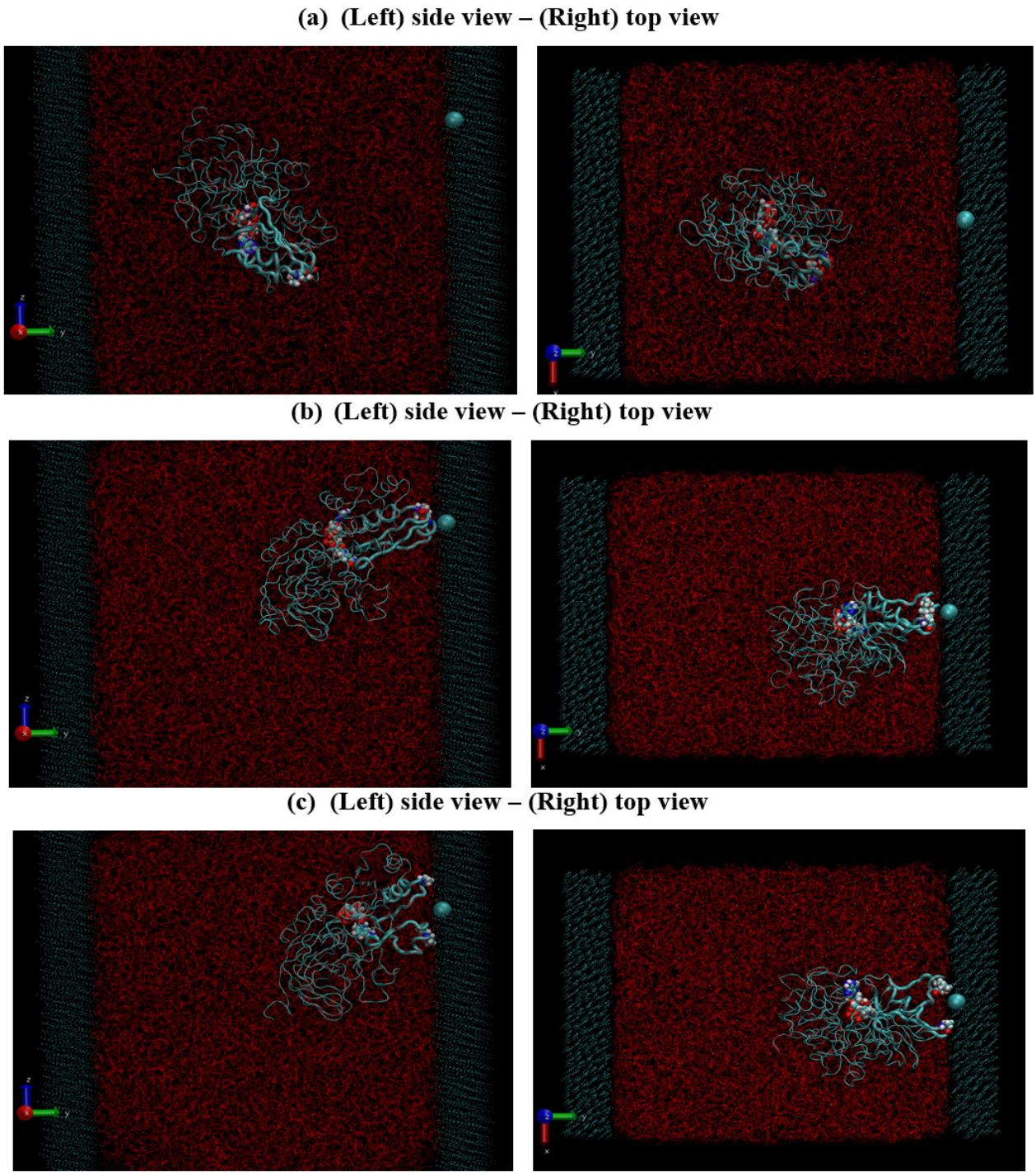


Figure 14 (a-c): The behavior of the enzyme' catalytic loops during the ADH+NAD adsorption on the graphitic carbon surface for: (a) - initially relaxed state, (b) - intermediate and (c) - final 100 ns equilibrium states.

Loop1 (res.280-res.300)

---GLU280-THR281-MET282-LYS283-ALA284-ALA285-LEU286-ASP287-LYS288-THR289-THR290-ALA291-GLY292-TRP293-GLY294-SER295-CYS296-THR297-PHE298-ILE299-GLY300---

Loop2 (res.307-res.327)

---GLY307-LEU308-THR309-VAL310-PHE311-PRO312-GLU313-GLU314-LEU315-ILE316-ILE317-GLY318-ARG319-THR320-ILE321-ASN322-GLY323-THR324-PHE325-PHE326-GLY327---

Conclusion

In summary, the adsorption process of the alcoholdihydrogenase (ADH) enzyme with its co-factor Nicotinamide Adenine Dinucleotide (NAD) on a graphite-like surface (C-surface) are analyzed experimentally and simulated by the MD (molecular dynamics) simulation method. The ADH+NAD enzyme are surrounded by water molecules and other chemical particles of the buffer system, which are constantly in thermal motion and colliding with a protein globule, they provide its chaotic linear and rotational

motion (similar to Brownian motion), as well as conformational motion. When entering the physical field of the sorbent surface, the protein molecule begins to interact with the C-surface. Due to the heterogeneity of the protein surface and its structure in general, rotational moments arise that lead to a certain orientation of the protein globule relative to the surface even before the formation of a protein-surface "complex" (including several active sorption centers for low-molecular-weight sorbates). This picture of protein sorption is fully consistent with modern ideas about the dynamic nature of the process with constant conformational transitions (conformational "breathing" of the protein structure) and the movement of the entire protein structure in space. When a protein molecule enters the physical field of the sorption surface, which causes electrostatic and a set of van der Waals interactions, already on approaching the surface the protein globule is oriented in the surface field, leading to a decrease in the free energy of the system. After landing the globule on the sorbent surface, lateral movement and rotation of the protein molecule can be carried out with a search for the global minimum interaction energy corresponding to the sorption equilibrium.

Subsequently, the MD numerical experiment realized in current study using the AMBER-18 package (fast module "pmemd. cuda" implementation), provides the mapping of the orientational adsorption of ADH enzyme with an essential extension of the original basic model, thereby representing the orientation of the titratable amino acid residues of ADH in detail. Detection of a stable orientation of key titratable amino acids may be a necessary step in the next studies and experiment realization, to be carried out by varying pH values and water-salt balance. In the future, this discovery may clarify the ways of surface modification with positively or negatively charged groups, thereby simulating the properties of real sorbents used for protein immobilization.

In the above figures, Figure 10, 12 & 14 the ADH+NAD molecule begins to "spin" in search of the optimal orientation. The results of large-scale 100ns long molecular dynamics calculations (billions time-steps of the integration of dynamic equations of motion) realized the slow interacting processes and orientation adsorption of the ADH+NAD molecule with the sorbent C-surface that show how the ADH+NAD molecule begins to rotate around the z axis counterclockwise, as a result of which it is rotated by its the catalytic cavity in the ADH center (presumably where a slit of ADH cofactor NAD is located) perpendicular to the surface, as shown in Figures 10-14. In the case under consideration, such orientation is possible due to hydrophobic interactions between the protein molecule and the C-surface. At the end of the dynamical changes, the ADH+NAD globule orientation (Figures 10-14) has to correspond to the direction of the Z axis towards the surface of the graphite-like sorbent (C-surface). It should be noted that this orientation of the ADH globule was predicted by Prof. Gladyshev P.P. with colleagues [7-10], who carried out numerical modeling of the process of oriented sorption of ADH on a hydrophobic surface using a simpler approach that does not take into account some changes in the conformation of this enzyme during the transition from the crystalline state to solution. Thus, we can assume that the coincidence of the calculated conformation of ADH on a hydrophobic surface in our case and in the earlier work of Gladyshev P.P. mutually confirms the correctness of both approaches to calculating oriented sorption in the system under consideration [7-10].

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