NANOSYSTEMS:
PHYSICS, CHEMISTRY, MATHEMATICS

Original article

Baigunov I.A., et al. *Nanosystems: Phys. Chem. Math.*, 2025, **16** (2), 192–198.
http://nanojournal.ifmo.ru
DOI 10.17586/2220-8054-2025-16-2-192-198

Alcohol dehydrogenase: molecular dynamics study of conformational and orientational behaviour of the enzyme in complex with nad during sorption on the surface of electrode materials using graphite as an example

Ivan A. Baigunov[1,a], Kholmirzo T. Kholmurodov[1,2,3,4,b], Pavel P. Gladyshev[1,c]

[1]Dubna State University, Department of Chemistry, New Technologies and Materials, Dubna, Russia
[2]Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Russia
[3]Lomonosov Moscow State University, Department of Fundamental Nuclear Interactions, Moscow, Russia
[4]S. U. Umarov Physical-Technical Institute (PhTI), Dushanbe, Republic of Tajikistan

[a]vanek1997fev@yandex.ru, [b]kholmirzo@gmail.com, [c]pglad@yandex.ru

Corresponding author: Ivan A. Baigunov, vanek1997fev@yandex.ru

PACS 02.70.Ns, 36.20.-r

ABSTRACT In this work, computer molecular dynamics (MD) studies of the orientation and structural conformations of the alcohol dehydrogenase enzyme (hereinafter ADH) in complex with nicotine adenine dinucleotide (hereinafter NAD) during sorption on the surface of electrode materials using graphite as an example were carried out.

KEYWORDS alcohol dehydrogenase, molecular dynamics

ACKNOWLEDGEMENTS ...

FOR CITATION Baigunov I.A., Kholmurodov Kh.T., Gladyshev P.P. Alcohol dehydrogenase: molecular dynamics study of conformational and orientational behaviour of the enzyme in complex with nad during sorption on the surface of electrode materials using graphite as an example. *Nanosystems: Phys. Chem. Math.*, 2025, **16** (2), 192–198.

1. Introduction

The current study is devoted to the structural aspect of the ADH (alcohol dehydrogenase) enzyme and its co-enzyme NAD (Nicotinamide-Adenine-Dinucleotide) [1–5]. As shown by X-ray crystallography, the ADH enzyme undergoes global conformational changes, including rotation of the catalytic domain relative to the coenzyme binding domain and rearrangement of the active center to obtain a catalytically active enzyme. The conformational change requires a complete coenzyme and depends on various chemical or mutational substitutions that can increase the catalytic activity due to changes in the isomerization kinetics and the rate of dissociation of coenzymes [2–6]. As for the structural aspect of the enzyme, using experimental observations and mathematical modeling of the protein, the orientation of ADH on various sorbents and conductive matrices were studied depending on the pH of the solution. As is known, deactivation of the enzyme caused by unsuitable conditions (temperature, pH), thereby introducing a change in the activity of the ADH enzyme. The implementation of various options includes, for example, the immobilization of a two-substrate enzyme on the surface of electrode materials [7–15]. However, it should be noted that the experimental study of the above issues is difficult. Therefore, in recent years, computational and simulation analysis methods have been widely used for these purposes [16–23]. Molecular dynamics (MD) modeling is currently widely used with many software packages designed for MD modeling (the DL_POLY, which contains and programmed all the potentials necessary for construction; the AMBER software package for simulating protein structures, Table 1).

Molecular dynamics simulation involves a series of steps, shown in Fig. 1.

In this work, we used computer molecular dynamics (MD) modeling to study the structural and conformational changes of the ADH enzyme with its cofactor NAD occurring in an aqueous solution interacting with the surface of the electrode material. Graphite serves as the surface and the MD analysis data allow studying the changes in the ADH + NAD structural conformations on atomic-molecular level in detail.

TABLE 1. Potentials used in the AMBER software package

Potential	Purpose
$U(R) = \sum_{\text{bonds}} K_r(r - r_{eq})^2$	Harmonic potential for two atoms
$U(R) = \sum_{\text{angles}} K_\theta(\theta - \theta_{eq})^2$	Harmonic potential for angular bond of three atoms
$U(R) = \sum_{\text{duhedrals}} \frac{V_n}{2}(1 - \cos[n\varphi - \gamma])$	Harmonic potential for four atoms
$U(R) = \sum_i \sum_j \left[\left(\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} \right) + \frac{q_i q_j}{R_{ij}} \right]$	Lennard–Jones potential (van der Waals interactions) and electrostatic interaction

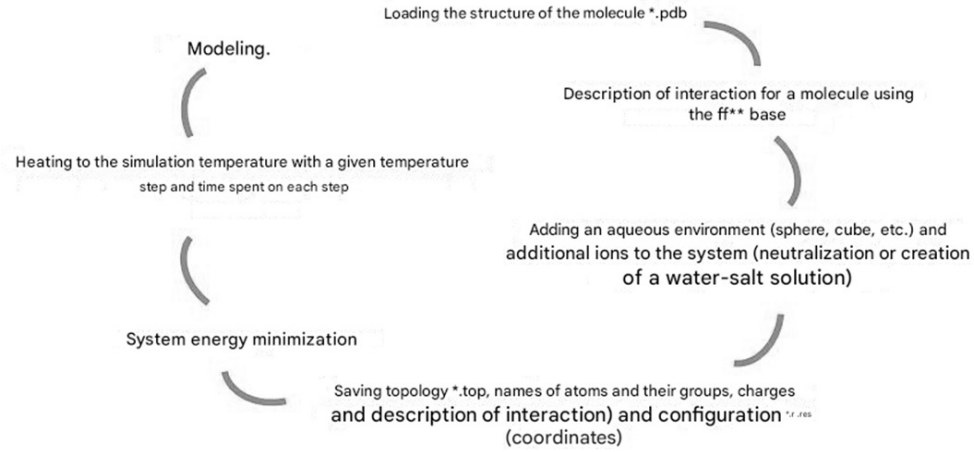
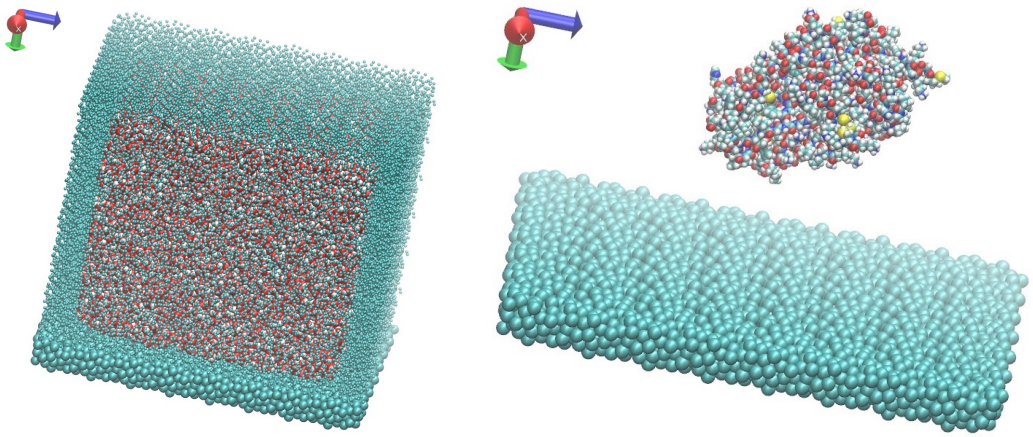


FIG. 1. Main stages of the numerical experiment

2. Experimental part

In this section, we present the descriptions of the main parameters and algorithms used in the computational molecular dynamics (MD) simulations. In this study, we used both CPU and GPU-based computing environments to perform the MD simulations using the AMBER package. We used multiple computing environments such as a 16-core cluster, Geforce GPU (GPU = (GTX 1080 Ti)) to implement the MD simulations in Amber 18 (pmemd; MD/AMBER) with GPU acceleration (pmemd.cuda). We refer to the Amber 2018 program code and reference manual [3, 16–18]. The calculations were carried out on the servers of the Heterogeneous Platform "HybriLIT" of the Multifunctional Information and Computing Complex (MIC), MLIT (M.G. Meshcheryakov Laboratory of Information Technologies), JINR (Joint Institute for Nuclear Research). The heterogeneous platform consists of the supercomputer "Govorun" and the educational and test site "HybriLIT" at JINR, Dubna, and on the local server of the Frank Laboratory of Neutron Physics (LNP) of JINR. We have implemented the main production MD simulations (CPU / GPU) (common also with many other simulation types) for PDB ID: 3COS crystal structure of human alcohol dehydrogenase class II (ADH) [19]. In this work, MD simulations were performed with the Amber 18 code (CPU/GPU environment). The MD simulations on the molecular system ADH + NAD + water + carbon surface (Fig. 2) were performed in three steps: energy minimization, NVT and NPT relaxation procedures. As for NVT: The canonical ensemble, where the system is kept from changes in moles (N), volume (V), and temperature (T). This set-up is also known as constant-temperature molecular dynamics, and requires a thermostat. The NPT: The isothermal-isobaric ensemble, where the system is kept from changes in moles (N), pressure (P), and temperature (T). Both a thermostat and barostat are needed. Due to the Amber 18 code' capabilities we used the Langevin Dynamics (NVT and NPT) to attempt to mimic solvent viscosity by introducing things that occasionally cause friction and perturb the system. When used to control temperature, a small damping constant, γ, should be used. In the Berendsen Thermostat: the system is weakly coupled to a heat bath at a set temperature. The thermostat doesn't



(a) (b)

FIG. 2. General molecular design of the system ADH + NAD + water + graphite surface

mirror the canonical ensemble for small systems, but large systems are roughly ok. It uses a leap-frog algorithm to rescale velocities of particles, controlling temperature.

The MD simulations in Amber18 with CPU/GPU acceleration (pmemd/pmemd.cuda) were performed for three main setup phases that contain the main MD production:

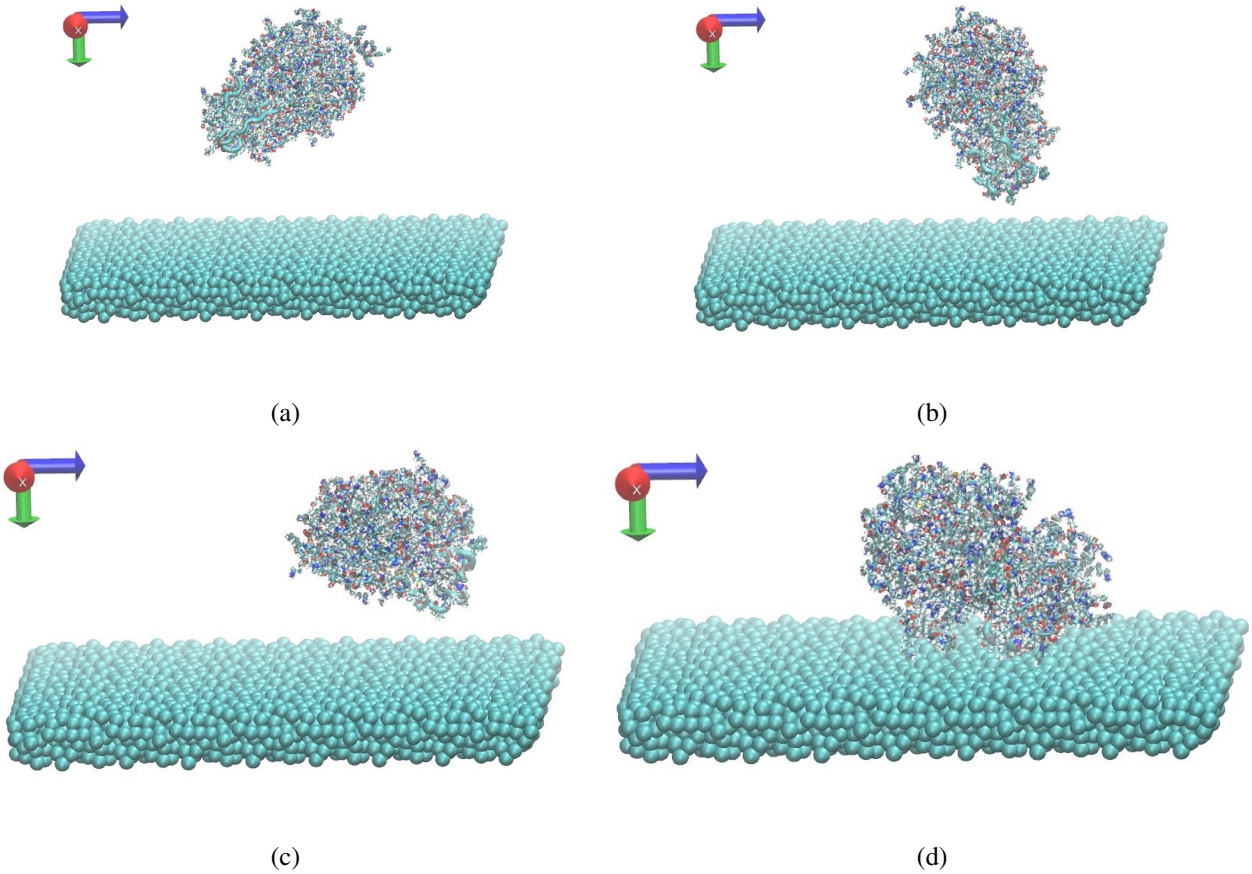
1) Minimization of the system to reduce bad contacts;
2) Slowly heat the system to the target temperature;
3) Equilibration of the system at the target temperature.

In the first step, we minimized the system by applying constraints to the backbone atoms and to some atoms. The minimization was performed using sander instead of pmemd to ensure and evaluate the behavior of the energies during the minimization step. We then ran constant-volume heating simulations on the minimized structures to slowly heat the system from 10 to 303 K over some initial 2.0 ns steps of the simulation with a target temperature of 303 K. We retain the constraints on the backbone atoms, but with a weaker force constant than that used during the minimization. These simulations were performed using pmemd, pmemd.MPI, sander, or sander.MPI instead of using the GPU-accelerated code with pmemd.cuda. At the equilibration step, we equilibrate the system (ADH + NAD + surface + water) at a target temperature of 303 K.

3. Results and discussion

In Fig. 3(a–d), we present the obtained images of the ADH + NAD adsorption process on the graphitic carbon surface during long-term 100 ns dynamic changes from (a) the initially relaxed state to (b–c) intermediate states and (d) the final equilibrium state. The ADH + NAD enzyme underwent multimillion-second conformational and rotational changes before adsorption on this graphitic carbon (C-surface) to be finally trapped and relaxed on the surface. The dynamics of the ADH + NAD adsorption behavior on the graphitic carbon surface was monitored using MD/AMBER calculations and Visual Molecular Dynamics (VMD) software. Fig. 3(a–d) presents the results of MD calculation with (ntb=1) periodic boundaries of constant volume when minimizing and initially heating/equilibrating the whole system, ADH + NAD + aqueous solvent + graphite surface, and with (ntb=2) periodic boundaries of constant pressure when used for the production run after we heated and equilibrated at constant volume.

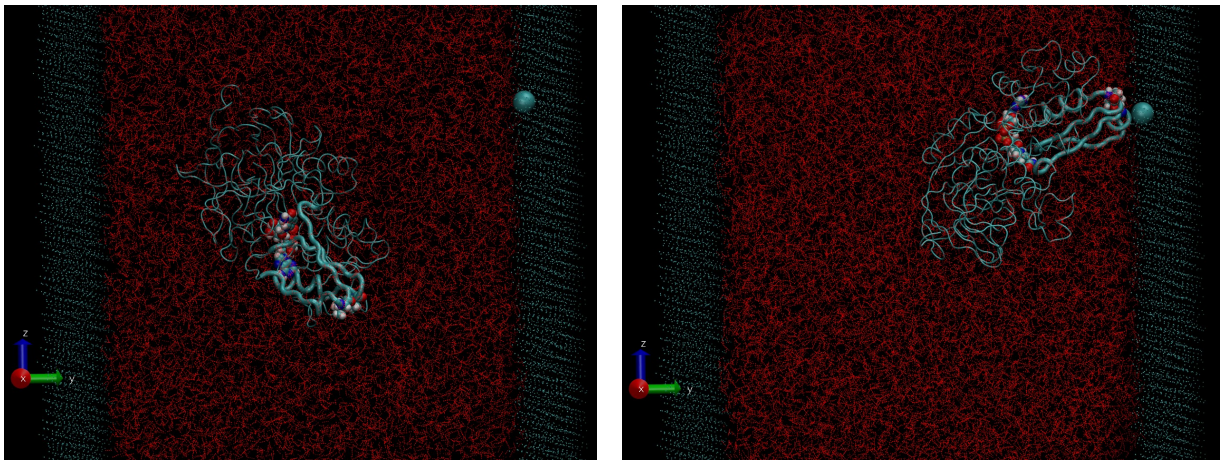
It is worth noting that finding the relaxed equilibrium of the ADH + NAD + water + surface system is a slow process, so far for each set of MD simulations we have performed 100 ns of calculations using the extremely fast module “pmemd.cuda”. One of the non-trivial events in the conformational structural dynamics of the whole ADH + NAD + water/graphite system and tracking of individual amino acid residues should be the behavior of the catalytic loops of the enzyme. Fig. 4(a–b) shows the dynamic patterns of the ADH + NAD/C-surface and the adsorption processes accompanied by gradual changes in the orientation of the two catalytic loops of the ADH + NAD molecule relative to the graphite surface. An important observation is that these two catalytic loops are located close inside the ADH + NAD molecule, upon reaching the adsorbing graphite surface we can see the separation of these loops from each other. The key summary of the whole process, as shown in Fig. 3(a–d), should be the separation from each other and hence the opening of the important catalytic loops of ADH + NAD due to the influence of the adsorbing C-surface of these two enzyme loops.



(a) (b)

(c) (d)

FIG. 3. Adsorption of ADH + NAD on graphite (C-surface) during 100 ns of dynamic and conformational changes



(a) (b)

FIG. 4. The ADH + NAD orientation together with the position of the catalytic loops of ADH + NAD on the graphite surface of carbon

Next, Fig. 5 shows the arrangement of the two above-mentioned catalytic loops of the ADH + NAD molecule relative to the graphite surface. Here, the two terminal amino acid residues THR290 and ILE317 with the separations between these two terminal amino acids is shown.

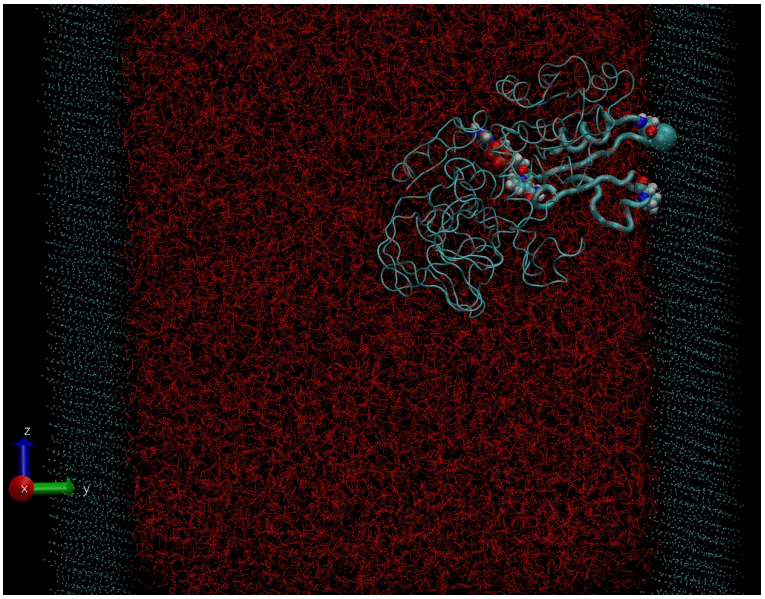


FIG. 5. Position of the catalytic loops of ADH + NAD on the graphite surface of carbon (a). Dynamics of the distance between the catalytic loops of ADH + NAD depending on time (b)

The MD results presented in Fig. 5 for the arrangement of the catalytic loops during the adsorption of the ADH + NAD molecule on the graphitic carbon surface in the final (100 ns) state of time clearly to confirm the important observation mentioned above that the two catalytic loops separate from each other upon reaching the adsorbing graphite surface, whereas they were initially located close to each other inside the ADH + NAD molecule. The above observation and the presented data on the dynamics of the ADH + NAD catalytic loops on the graphitic carbon surface correlate with the dynamic changes and rotations of the NAD coenzyme inside the ADH molecule. Using the data in Figs. 4–5, we observe the conformational changes of NAD upon ADH adsorption on the surface and the open gap between the catalytic loops. In Fig. 6, we have presented the positions of the atoms at the NAD chain, as well as the atoms in the central region of NAD. Fig. 6 shows the structure of the NAD molecule which correlate with the dynamical changes shown in Figs. 4–5 above.

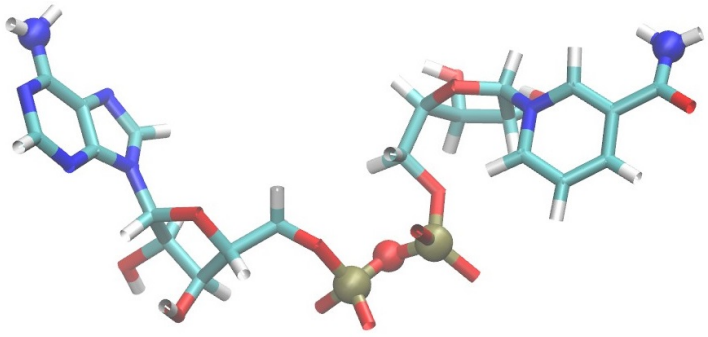


FIG. 6. The NAD structure with entered points correlates with dynamical changes in the ADH

It is worth noting that the current observations correlate with some experimental works related to the immobilization and adsorption of ADH enzyme on the carbon surface, the location of the enzyme and its fixation on the carbon platforms. It is seen that the dynamic changes of γ over time completely correlate with the dynamics of the change in the distance between the catalytic loops of ADH + NAD on the graphite surface. The comparison of the results in Figs. 4–5 are the key features of the entire process of ADH + NAD/C-surface adsorption, which is accompanied by a nontrivial structural transformation of the NAD coenzyme, correlating with the behavior of the catalytic loops of the ADH enzyme. The above observation and the presented data on the dynamics of the ADH + NAD catalytic loops on the graphite surface

correlate with the dynamic changes and rotations of the NAD coenzyme inside the ADH molecule. Moreover, the results also indicate that in order to maintain the environment in which the ADH enzyme exhibits good activity and to provide conditions for future technological applications, physiological conditions and ambient temperature can be satisfactorily applied to the enzymatic system including the dehydrogenase enzyme. At the same time, the choice of carbon surfaces and platforms is motivated by good control of the enzyme arrangement on the surface at a very low enzyme consumption. The kinetic rates obtained for the ADH enzyme attached to the carbon surface indicate a decrease in activity after the immobilization and fixation process, a significant loss of enzymatic activity observed after immobilization, although the affinity between the enzymes and their substrates and coenzymes is preserved.

4. Conclusion

In summary, the structural conformations of the alcohol dehydrogenase (ADH) enzyme with its cofactor nicotinamide adenine dinucleotide (NAD) were studied on a graphite surface (C-surface) using the MD (molecular dynamics) simulation method. The ADH + NAD enzyme was simulated in an aqueous environment, the molecules of which are constantly in thermal motion and collide with the protein globule, thereby providing chaotic linear and rotational motion, as well as conformational movements. Subsequently, the numerical MD experiment implemented in the current study using the AMBER-18 package (implementation of the fast module "pmemd.cuda") provides useful statistics regarding the most interesting aspects of the structural study of ADH related to the conformational changes of ADH, including the rotation of the catalytic domain, the coenzyme binding domain, the rearrangement of the active site, etc. The above analysis is important for understanding the atomic/molecular details of the catalytically active enzyme, so far the long dynamic simulation of 100 ns allows tracking the conformational and rotational changes of the ADH + NAD system in aqueous medium.

References

[1] Wagenknecht P.S., Penney J.M., Hembre R.T. Transition-metal-catalyzed regeneration of nicotinamide coenzymes with hydrogen. *Organometallics*, 2003, **22** (6), P. 1180–1182.

[2] Nakamura K., Yamanaka R. Light mediated cofactor recycling system in biocatalytic asymmetric reduction of ketone. *Chemical Communications*, 2002, **16**, P. 1782–1783.

[3] Bilan D.S., Belousov V.V. Genetically encoded probes for NAD+/NADH monitoring. *Free Radical Biology and Medicine*, 2016, **100**, P. 32–42.

[4] Presečki A.V., Vasić-Rački D. Modelling of the alcohol dehydrogenase production in baker's yeast. *Process biochemistry*, 2005, **40** (8), P. 2781–2791.

[5] Tušek A., Šalić A., Kurtanjek Ž., Zelić B. Modeling and kinetic parameter estimation of alcohol dehydrogenase-catalyzed hexanol oxidation in a microreactor. *Engineering in Life Sciences*, 2012, **12** (1), P. 49–56.

[6] Orlich B., Berger H., Lade M., Schomäcker R. Stability and activity of alcohol dehydrogenases in W/O-microemulsions: Enantioselective reduction including cofactor regeneration. *Biotechnology and Bioengineering*, 2000, **70** (6), P. 638–646.

[7] Höhn S., Zheng K., Romeis S., Brehl M., Peukert W., de Ligny D., Boccaccini A.R. Effects of medium pH and preconditioning treatment on protein adsorption on 45S5 bioactive glass surfaces. *Advanced Materials Interfaces*, 2020, **7** (15), 2000420.

[8] Benavidez T.E., Torrente D., Marucho M., Garcia C.D. Adsorption and catalytic activity of glucose oxidase accumulated on OTCE upon the application of external potential. *J. of colloid and interface science*, 2014, **435**, P. 164–170.

[9] Wang F., Zhang Y.Q. Bioconjugation of silk fibroin nanoparticles with enzyme and peptide and their characterization. *Advances in protein chemistry and structural biology*, 2015, **98**, P. 263–291.

[10] Welborn V.V. Structural dynamics and computational design of synthetic enzymes. *Chem. Catalysis*, 2022, **2** (1), P. 19–28.

[11] Norde W., Lyklema J. Why proteins prefer interfaces. *J. of Biomaterials Science, Polymer Edition*, 1991, **2** (3), P. 183–202.

[12] Andrade J.D. (Ed.). *Surface and interfacial aspects of biomedical polymers*, 1985, Plenum Press, New York, 1985, **1**, P. 249–292.

[13] Gladyshev P.P., Shapovalov Yu.A., Kvasova V.P. *Reconstructed oxidoreductase systems*, Science, Alma-ata, KazSSR, 1987, 187 p.

[14] Gladyshev P.P., Goriaev M.I., Shpil'berg I.G., Iu A.S. Sorption immobilization of NAD-dependent enzyme systems. I. Influence of electrostatic interactions on the orientation of alcohol dehydrogenase on the sorbent surface. *Molekuliarnaia Biologiia*, 1982, **16** (5), P. 938–942. (in Russian).

[15] Gladyshev P.P., Goriaev M.I., Shpil'berg I.G. Sorption immobilization of NAD-dependent enzyme systems. II. Influence of hydrophobic interactions on the orientation of alcohol dehydrogenase on the sorbent surface. *Molekuliarnaia Biologiia*, 1982, **16** (5), P. 943–947. (in Russian).

[16] Foresman J., Frish E. *Exploring chemistry*, Gaussian Inc., Pittsburg, USA, 1996, **21**, P. 93–123

[17] Leach A.R. Molecular modelling: principles and applications. *Pearson education*, Harlow, 2001, 727 p.

[18] Case D.A., Cheatham III T.E., Darden T., Gohlke H., Luo R., Merz Jr. K.M., Woods R.J. The Amber biomolecular simulation programs. *J. of Computational Chemistry*, 2005, **26** (16), P. 1668–1688.

[19] Case D.A., Aktulga H.M., Belfon K., Cerutti D.S., Cisneros G.A., Cruzeiro V.W.D., Merz Jr. K.M. AmberTools. *J. of Chemical Information and Modeling*, 2023, **63** (20), P. 6183–6191.

[20] Lee T.S., Cerutti D.S., Mermelstein D., Lin C., LeGrand S., Giese T.J., York D.M. GPU-accelerated molecular dynamics and free energy methods in Amber18: performance enhancements and new features. *J. of Chemical Information and Modeling*, 2018, **58** (10), P. 2043–2050.

[21] Cruzeiro V.W.D., Amaral M.S., Roitberg A.E. Redox potential replica exchange molecular dynamics at constant pH in AMBER: Implementation and validation. *The J. of Chemical Physics*, 2018, **149** (7).

[22] Kholmurodov K.T. *Models in bioscience and materials research: Molecular dynamics and related techniques*, Nova Science Publishers Ltd., New York, 2013, 208 p.

[23] Kholmurodov K.T. *Computational materials and biological sciences*, Nova Science Publishers Ltd., New York, 2015, 188 p.

Submitted 21 January 2025; revised 19 February 2025; accepted 20 February 2025

Information about the authors:

Ivan A. Baigunov – Dubna State University, Department of Chemistry, New Technologies and Materials, Universitetskaya, 19, Dubna, Moscow Region, 141980, Russia; ORCID 0009-0000-8380-6218; vanek1997fev@yandex.ru

Kholmirzo T. Kholmurodov – Dubna State University, Department of Chemistry, New Technologies and Materials, Universitetskaya, 19, Dubna, Moscow Region, 141980, Russia; Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Joliot-Curie, 6, Dubna, Moscow Region, 141980, Russia; Lomonosov Moscow State University, Department of Fundamental Nuclear Interactions, Faculty of Physics, Leninskiye Gory, GSP-1, Moscow, 119991, Russia; S.U. Umarov Physical-Technical Institute (PhTI), Aini ave., 299/1, Dushanbe, 734063, Republic of Tajikistan;
ORCID 0000-0002-9415-8276; kholmirzo@gmail.com

Pavel P. Gladyshev – Dubna State University, Department of Chemistry, New Technologies and Materials, Universitetskaya, 19, Dubna, Moscow Region, 141980, Russia; ORCID 0000-0002-7449-4475; pglad@yandex.ru

Conflict of interest: the authors declare no conflict of interest.