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Solvent-driven structural dynamics of NAD(P)H:FMN-oxidoreductase

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Motivation and Aim: To develop a highly sensitive indicator system for detecting trace pollutants, such as petroleum products, it is effective to utilize an enzymatic system that includes bacterial luciferase (BLuc) and NAD(P)H:FMN oxidoreductase (Red). Pollution levels can be measured by observing variations in the intensity of the light emitted during the bioluminescent reaction catalized by Bluc. When Bluc is coupled with Red, a continuous luminescent signal can be achieved, since Red provides the reduced substrate necessary for the luciferase reaction. Recent research indicates that the sensitivity of the BLuc-Red system to external influences is often linked to changes in Red activity [1]. Thus, exploring how different solvents used for probe preparation affect the structure and dynamics of this enzyme is important for optimizing its application in enzymatic biotests.
Methods and Algorithms: The structure of Red was taken from the Protein Data Bank (PDB ID: 1VFR), and the ligand molecules were removed before modeling. Two systems were then prepared: (1) containing the protein, water molecules, and Na^+ ions, and (2) the protein in an aqueous solution of acetone (w/w 10 %). For each system, energy minimization was performed, followed by two relaxation steps (NVT and NPT) and three independent runs of molecular dynamics. The modeling was conducted using the GROMACS 2020.4 software package in the GROMOS96 54a7 force field. The simulation time for each run was 100 ns.
Results: As a result of molecular dynamics simulations, it was found that an aqueous solution of acetone has little effect on the stability of the Red structure. Mobility analysis showed that the dynamics of certain amino acid residues in the flexible domain (from 90 to 134 a.a.) change in the presence of acetone. This region of the structure forms the binding site for one of the substrates of Red – NAD(P)H. Since the active center of the enzyme is formed by two subunits in a homodimer, a comparative assessment of hydrogen bonds between the subunits was conducted; however, no significant differences were found.
Conclusion: The presence of acetone in the reaction mixture may lead to changes in Red's specificity for this substrate and affect the efficiency of the catalyzed reaction, which should be taken into account when optimizing the reaction mixture for biotesting.
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References

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