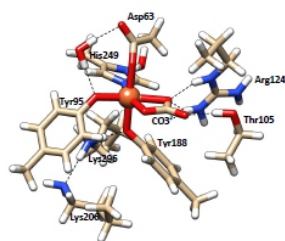## Healthcare & Biomaterial

5

Healthcare Research: Computer Aided Drug Design

Mahesh Sundararajan[1,2] **and A. K. Pathak***[1,2]

[1]*Theoretical Chemistry Section, Chemistry Division, Bhabha Atomic Research Centre (BARC), Trombay-400085, INDIA*
[2]*Homi Bhabha National Institute, Anushaktinagar, Mumbai-400094, INDIA*

Active site of iron-transferrin

ABSTRACT

A brief account of research related to healthcare carried out in recent years in our group is portrayed here. With the advent of supercomputers, robust statistical mechanics based methods, and fast theoretical algorithms, computational chemistry research now plays an unprecedented role in healthcare and have direct relevance to the programs of DAE. The understanding of physical principles underlying biological phenomena and biological processes that are not otherwise achievable through experiments is investigated by employing both large-scale all atom molecular dynamics simulations and quantum mechanical methods.

KEYWORDS: *Biomaterial, Heavy water, Drug design, Decorporation, Targeted drug delivery, Bio-nanocontainer*

Introduction

It is pertinent to pose a few questions, e.g., when and why a biomolecule (protein, RNA, DNA, etc.) folds, unfolds, or changes its conformation; why a drug stops working, etc. Answers to these questions can open up potentially important new avenues with immense healthcare, engineering, and societal applications. Successfully solving them might lead to the next super-drug or molecule with targeted, cost-effective applications in healthcare with minimal unwanted side-effects. However, the biological processes are so slow that studying them on the best supercomputers would take almost the age of the universe by employing conventional molecular dynamics simulation. One can overcome the uphill task by employing a biased simulation protocol. This will help in designing new decorporating agents for actinide contaminations, the use of heavy water in pharmacies, the design of drugs with better efficacy in reactivating intoxicated acetylcholine esterase, and the speciation of radionuclides in humic substances. In what follows, various codes used in biophysical research are described in Sec. 2. Various results, namely, thermo-stabilization of polio viral vaccine by using D_2O in Sec. 3.1, use of viral capsid as bio-nanocontainer in Sec. 3.2, mechanistic insight into designing decorporation agents for actinides in Sec. 3.3, reactivation of organophosporous intoxicated acetylcholinesterase in Sec. 3.4, interaction of fission products with humic substances in Sec. 3.5 and biospeciation of actinides with blood serum transferrin in Sec. 3.6 are depicted. Finally, a brief summary is presented in Sec. 4 as a conclusion.

Methodology

At present, *ab initio* electronic structure calculations for bio-molecules, and classical mechanics based all atom molecular dynamics simulations have been extensively used. GAMESS, TURBOMOLE and ORCA codes have been used for electronic structure calculations, in-house codes have been employed for analysis of mined data, and GROMACS has been used for all atom molecular dynamics simulations of biological systems to understand the observed phenomena and predict the mechanism of bio molecular action.

Results & Discussion

Thermostabilization of Polio Viral Vaccine by using D_2O

Heat plays a detrimental role in the therapeutic activity of many thermolabile liquid pharmaceuticals. This demands the maintenance of an uneconomical and logistically challenging cold chain, i.e., to maintain the pharmaceutical within a specified low temperature, and this is a billion-dollar industry. The majority of the live vaccines are stored at 2-8^{0}C in order to sustain optimal cold temperatures, accounting for 80% of the financial cost of vaccination. The oral polio vaccine is considered to be the most thermo-labile of all the common childhood vaccines. Despite heavy water (D_2O) having been known for a long time to stabilize attenuated viral RNA against thermo-degradation, the molecular underpinnings of its mechanism of action are still lacking. Whereas, understanding the basis of D_2O action is an important step that might reform the way other thermolabile drugs are stored and could possibly minimise the "cold chain" problem, thus making them more economical and reaching places with a scarcity of proper logistics. Here, using a combination of parallel tempering and well-tempered metadynamics, a biased MD simulation in both light water (H_2O) and D_2O, we have fully described the free energy surface associated with the folding or unfolding of a RNA hairpin of poliovirus-like enteroviruses. Parallel tempering meta-dynamics simulations reveal that in D_2O there is a considerable increase in the stability of the folded state as monitored through the intramolecular hydrogen bond, size, shape, and flexibility of RNA structures. This transforms into a higher melting temperature (T_m) in D_2O by 41 K (see Fig.1) when compared to that of light water (H_2O) [1]. Simulation in heavy water clearly showed that D_2O strengthens the HB network in the solvent, lengthens inter-residue water-bridge lifetime, and weakens the dynamical coupling of the hairpin to its solvation environment, which enhances the rigidity of solvent-exposed

*Author for Correspondence: A. K. Pathak
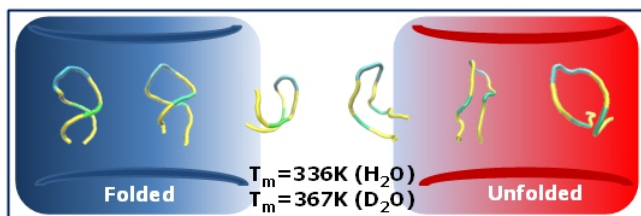E-mail: akpathak@barc.gov.in*

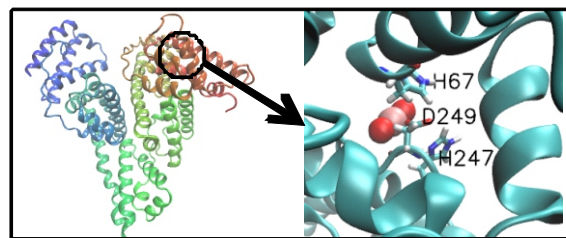Fig.1: Folding and unfolding behavior of RNA hairpin in D_2O along with respective melting temperatures.

Fig.2: Structure of the human serum albumin and its binding with UO_2^{2+}

sites of the native configurations. The results suggest that, like other added osmoprotectants, D_2O can act as a thermostabilizer when used as a solvent for storing polio vaccine. Liquid heavy water in place of light water has the tremendous potential to break the "cold chain" problem.

Viral Capsid as Bio-Nanocontainer

Viruses are the simplest biological systems, essentially composed of a protein shell or capsid that encloses the packaged genetic material. The infection process and release of packaged materials depend on the structural changes of the capsid, which are sometimes induced by thermal fluctuations. Thermal fluctuations that induce structural changes in a capsid play a major role in the delivery of the confined material. Characterising the successive structural changes as a function of temperature may provide fresh insights into antiviral and nanomaterial strategies. We have calculated the heat-induced changes in the properties of a virus capsid using large-scale $(3.0 \times 10^6$ atoms) all-atom MD simulations, which was accomplished in the CPU-GPU architecture of the BARC Anupam system in a year time – by far this is a national standard. We focus on two heat-induced structural changes of the viral shell, namely, the dynamical transition (DT) [2] and the breathing transition (BT) [3]. DT (222K) brings forth the flexibility in the shell, which ceased to exist at cryogenic conditions and is measured through the sudden and sharp changes in the collective motion of hydrogen atoms. It is observed that at BT temperature (318K), the capsid changes from a non-functional form to a flexible and functional state, such that the breathing motions of virus particles become large enough to initiate disassembly and infection process [3]. The distribution of equilibrium atomistic stresses in the peptide fragments of the capsid reveals a largely asymmetric nature and suggests that structural breathing may actually represent large dynamic changes in the hotspot regions, far from the capsid pore, which bears remarkable resemblance to the recently conducted hydrogen-deuterium exchange coupled to mass spectrometry experiment. The findings have possible fallout in the development of therapeutic inhibitors of viral shells in general, the design of novel bio-nanocontainers, improved vaccines, targeted drug delivery, inactivation procedures for viruses in the food and pharmaceutical industries, heat-induced sanitization of public places, etc.

Mechanistic Insight into Designing Decorporation Agents for Actinides

Radionuclides, mostly actinides are regularly handled in various routes of the nuclear fuel cycle, ranging from mining and power production to the reprocessing of spent nuclear fuel. Although there are stringent safety measures during the regular handling of radionuclides at all stages, the possibility of internal contamination due to these radionuclides cannot be ignored. Due to the growing applications of radionuclide-based materials in nuclear energy, defence, space industries, and medical applications, the danger of exposure to these

radionuclides is a mounting concern for human health. During the handling of actinides, accidental discharge into the environment can cause severe health risks to human beings, ranging from minor problems like nausea and vomiting to more severe effects like failure of vital organs, cancer, and even death due to both radiological and chemical toxicity. These radionuclides can enter the human body via four major pathways: inhalation, through a wound (including an accidental injection), ingestion, or absorption through intact skin. Certain radionuclides, e.g., uranium, exhibit long biological lifetimes and are excreted at variable rates from the body. Recently, it was shown that human serum albumin (HSA), a major zinc carrier protein, can also bind with uranyl ions (see Fig.2); however, their behaviour with different biological ligands remains obscure. It is interesting to mention that environmental factors like pH plays very important role in the dynamics of proteins [4-6]. Thus effect of pH is included to understand the metal ion binding with HSA. Our MD study reveals that uranyl ions cannot associate to the zinc ion bound HSA protein but can be captured by free HSA at all pH values, i.e. endosomal, alkaline, and physiological pH [7]. The findings will provide important contributions in designing potential candidates for the decorporation of actinides from the human body.

Reactivation of Organophosporous Intoxicated Acetylcholinesterase

In an effort to understand how a drug molecule accomplishes its task of interacting with protein molecules, several large-scale computations were performed and compared the efficacy of existing oxime drug molecules towards the recovery of the free nerve enzyme, acetylcholinesterase (AChE) from organophosporous (OP) or chemical warfare agent intoxications [8-9]. AChE is a serine hydrolase that catalyses the breakdown of the neurotransmitter acetylcholine. It hydrolyzes the choline ester into choline and acetic acid in order to terminate synaptic transmission in neuromuscular junctions and in chemical synapses of the cholinergic type, e.g., cholinergic brain synapses. AChE, the primary cholinesterase enzyme, is highly efficient, but its catalytic activity is limited by the diffusion of the substrate. The very high catalytic activity of AChE is attributed to a catalytic triad, which consists of a serine, a glutamate, and a histidine residue. The catalytic triad of the AChE is a vulnerable target of OP compounds [8-9]. The OP compounds bind to the serine of the catalytic triad and subsequently inhibit its catalytic activity. Despite the fact that fluorination makes a drug more lipophilic, the molecular level understanding of protein-fluorinated drug interaction is obscure [9-11]. Due to their enhanced ability to penetrate the blood-brain barrier, they are suitable for reactivation of OP-inactivated AChE in the central nervous system. Our MD studies show that the fluorinated oximes (FOBI/FHI-6) interact more strongly with the protein residues than the non-fluorinated oximes (OBI and HI-6); this is also verified from
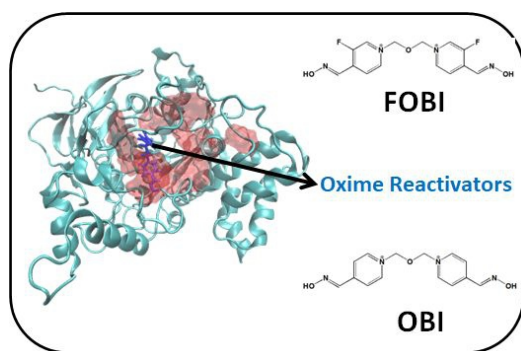
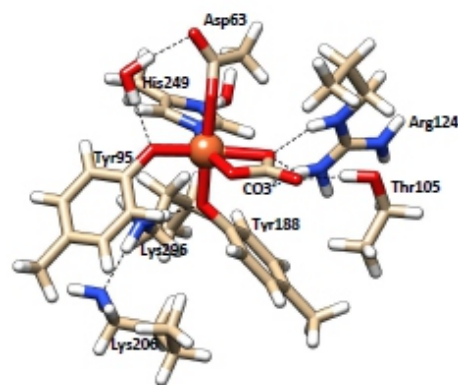Fig.3: Catalytic site of Acetylcholinesterase, and fluorinated and non-fluorinated oxime drugs.

Fig.4: Active site of iron-transferrin.

quantum mechanical calculations. Distinct unbinding pathways for FOBI/FHI-6 and OBI/HI-6 are observed, as evident from the potential of the mean force profiles (see Fig.3) of unbinding process [11-12]. It is suggested that FOBI/FHI-6 drugs are held more firmly in the gorge of the AChE in comparison to OBI/HI-6 and may lead to higher reactivation efficiency of the OP-inactivated enzyme [11-12].

Interaction of Fission Products with Humic Substances

Heavy metal ions such as uranyl (UO_2^{2+}), cesium (Cs^{137}) and strontium (Sr^{90}) radionuclides are one of the high level wastes generated from the back end of nuclear fuel cycle. The migration of these radionuclides in soil largely depends on chemical and biological reactivity of soil components. Natural Organic matter (NOM) is one of its essential components which are known to modulate the mobility of radionuclide cations like Cs^+ and Sr^{2+}. Humic (HA) and fulvic acids (FA) with varying oxygen contents can interact with heavy metal ions. HA itself can have few amino acids in their macromolecular structure. The NOM has several binding sites and identifying the probable binding pockets is a humongous task. To shed light on the possible binding sites and their transport behavior at the molecular level multi-scale model approach is used. We have used metadynamics, MD simulations and density functional theory based calculations to understand the binding mechanism of Sr^{2+} and Cs^+ cations to FA, a model for NOM [13-14]. We find that Sr^{2+} binds stronger through inner sphere mechanism, whereas Cs^+ binds weaker with outer sphere mechanism. These variation lead to Cs^+ is ready for plant uptake even in the presence of humic substances, whereas Sr^{2+} does not. Our simulations reveal that water molecules modulate the speciation of Sr^{2+} and Cs^+ ions. Very recently, we have reported the structures and binding of iodine species to HA. Iodine exist in two anionic forms namely iodide and iodates [15]. We proposed that iodate binds stronger than iodide and the former binding is less pH dependent than that of iodide. Our theory driven experiments based on UV-vis spectroscopic measurements of iodide and iodate with HA proved the computational predictions are indeed true.

Biospeciation of Actinides with Blood Serum Transferrin

Blood serum Transferrin (sTf) metalloprotein mobilizes iron to the cell. The Fe(III) ion is surrounded by carbonate, asparate, tyrosines and histidine as shown in Fig.4 [16]. The active site is strongly specific to Fe(III).

Only 30% of the protein is saturated with iron, whereas the remaining 70% is ready for binding other metal ions including actinides. Radionuclides such as Th, Cm, Pu ions are known to bind transferrin, but their geometric structures are not known [17-19]. The bio-speciation of actinides in transferrin is challenging due to the following reason. The coordination number for Fe(III) is six, whereas for An(III) or Ln(III) is larger than six. Thus, transferrin is tailer made for Fe(III), whereas for Ln and An, the coordination number needs to be expanded. These variations can be scrutinized with multi-scale modeling techniques that were performed here. The predicted octa-coordinated structure of Th(IV) and hepta-coordinated Cm(III) species are comparable with the EXAFS data [17-19]. The vacant coordination number of actinides is saturated by solvent water molecules which are only possible through polarizable water force-field parameters implemented in our MD simulations. The effects of pH are also investigated in detail with quantum chemical and MD simulations.

Further, the possible decorporation of radionuclides using HOPO and CAM based decorporating agents are investigated in detail [18]. We have taken Pu(IV) ion as an example to understand the Pu–sTf interaction is a primary step toward future design of its decorporating agents. Due to the obvious experimental difficulties, such as, handling of radionuclides associated with health and safety concerns, we have explored the use of multi-scale computational techniques to understand the Pu(IV) binding with sTf and look out for its decorporation at extracellular pH using suitable ligands. Until now, we have screened two decorporating agents for Pu(IV); hydroxypyridinone (HOPO) and catechol (CAM)-based ligands. Pu(IV) at protein binding site is found to be strong that it was not detached with the docked HOPO, whereas CAM is found to facilitate dislodging the heavy ion from the protein's binding influence.

Conclusion

In this article, theoretical research essential to (i) understand observed biological phenomena, (ii) predict mechanisms of bio-molecular action, and (iii) design new drugs with better efficacy is briefly discussed. The present results suggest the importance and necessity of the use of advanced statistical mechanics based strategies and sophisticated computational codes in a synchronised manner. The present-day amalgamation of the existing state-of-the-art computational techniques with artificial intelligence and machine learning-based algorithms will open up a new possibility in all facets of healthcare research in near future.

Acknowledgements

References

[[1]   A.K. Pathak, T. Bandyopadhyay,J. Chem. Phys., 2017, 46, 165104.

[2]   A.K. Pathak, T. Bandyopadhyay, J. Chem. Theory Comput., 2019, 15 , 2706.

[3]   A.K. Pathak, T. Bandyopadhyay, J. Biomole. Struct. Dyn. 2022, 40, 11900.

[4]   A.K. Pathak, Biopolymers, 2015, 103, 148.

[5]   A.K. Pathak, Rsc. Adv., 2015, 5, 94926.

[6]   A.K. Pathak, J. Phys. Cond. Matt., 2018, 30, 195101.

[7]   V. Mishra, A.K. Pathak, T. Bandyopadhyay, J. Biomole. Struct. Dyn. 2023, 41, 7318.

[8]   A.K. Pathak, T. Bandyopadhyay, Proteins ,2014, 82, 1799.

[9]   A.K. Pathak, T. Bandyopadhyay, Biopolymersm, 2016, 105, 10.

[10]   A.K. Pathak, T. Bandyopadhyay, J. Phys. Chem. B , 2015, 119 , 14460.

[11]   A.K. Pathak, T. Bandyopadhyay, Phys. Chem. Chem. Phys.,2017, 19, 5560.

[12]   A.K. Pathak, T. Bandyopadhyay, J. Phys. Chem. B, 2018, 122, 3876.

[13]   B. Sadhu, M. Sundararajan, T. Bandyopadhyay, J. Phys. Chem. B. 119 (2015) 10989.

[14]   M. Sundararajan, G. Rajaraman, S.K. Ghosh, Phys. Chem. Chem. Phys. 13 (2011) 18038.

[15]   M. Sundararajan, J. Ramkumar, A. Rasu, P. Venuvanalingam, Austin Environ. Sci. 8 (2023) 1093.

[16]   L. Mishra, M. Sundararajan, J. Chem. Sci. 131 (2019) 15.

[17]   L. Mishra, M. Sundararajan, T. Bandyopadhyay, Protein, 29 (2020) 193.

[18].   L. Mishra, M. Sundararajan, T. Bandyopadhyay, J. Biol. Inorg. Chem. 25 (2020) 213.

[19]   L. Mishra, P.D. Sawant, M. Sundararajan, T. Bandyopadhyay, J. Phys. Chem. B. 123 (2019) 2729.