Biophysics in Africa - 2023

Monday 25 September 2023 - Friday 29 September 2023

Book of Abstracts

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Computational biophysics / **85**

Discovery and Characterization of Cancer Mutations on DNA Transaction Enzymes

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We have developed a comprehensive approach to find and characterize the impact of cancer mutants on target proteins, and have applied it to DNA modification enzymes. This approach is an extension of conventional genome wide–association studies (GWAS). Our approach employs a new method for discovery and statistical validation of single nucleotide polymorphisms (SNPs) on specific genes called HyDn–SNP–S, followed by atomistic simulations via molecular dynamics (MD) and/or quantum mechanics/molecular mechanics (QM/MM) techniques. We will present the details of our mutant discovery and characterization approach, as well as examples of characterization of cancer mutations on DNA modification enzymes. Our simulations provide insights at the atomic level about how these mutations affect protein structure and/or function. Furthermore, experimental results validating our predictions will be presented.

Mathematical Biology / **86**

Travelling waves in inhomogeneous DNA system using Sine-Gordon equation

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An impulse function which is as a result of an external factor was considered as a complex dynamic system which has a nonlinear perturbations on the DNA system. This is used to describe the distortion that occurs within the DNA system. In describing the internal dynamics,mathematical model known as the sine-Gordon equations was employed. The equation describes angular oscillations of nitrous bases of the chain. The sine-Gordon model was modified to depictthe dynamics of the double helix of a DNA system. The proposed model was based on the inhomogeneities that exist in the base sequence of the DNA structure. Various works that has been done in similar areas were discussed as well as the method that was used. The effect of dissipation on the DNA was considered. Computer simulations were performed on the model to see the distortions that occurs in the DNA system

Electrophysiology / **87**

Automated Patch Clamp Evaluation of Snake Neurotoxins and Recombinant Antivenoms

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Snakebite was reinstated as an official Neglected Tropical Disease (NTD) by the World Health Organization in 2017, as it causes more than 100,000 deaths and around 400,000 amputations every year. Every snake species has a unique venom composition and consists of several dozen different toxins. The century-old technique to generate conventional antivenoms involves immunization horses with snake venoms, followed by purification of polyclonal antibodies from the horse blood plasma. However, such antivenoms are associated with several drawbacks related to equine-human immunoreactivity and adverse reactions, batch-to-batch variation, and high cost.
In the last decade, advances in antibody engineering have made antibody discovery and development more efficient, and it is now possible to develop recombinant antivenoms based on monoclonal antibodies targeting key venom toxin. One of the most medically relevant groups of snake toxins are the α-neurotoxins, which target nicotinic acetylcholine receptors (nAChRs).
For over two decades, automated patch clamp (APC) systems have been used to advance our understanding of ion channel biophysics, pharmacology, and their roles in physiology and disease.
Here, using QPatch II and Qube 384 APC, we functionally evaluated snake venom α-neurotoxins and toxin-neutralising IgG monoclonal antibodies (mAbs) on the muscle-type α1-nAChR.
This study demonstrates the potential of a range of IgGs to neutralize α-neurotoxins from several snake species. This is a critically important step towards enabling the design of novel, broadly-neutralizing recombinant antivenoms against snakebite envenoming.
This work highlights the potential and advantages of using high-throughput electrophysiology systems to evaluate the functional activity of protein-based toxins and antibodies.

Molecular Biophysics / 88

Recombinant expression and biophysical characterization of NAD-binding domain of S. mansoni Glyceraldehyde 3-phosphate dehydrogenase.

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Schistosomiasis is a devastating parasitic disease affecting over 200 million people globally and has the highest morbidity and mortality in sub-Saharan Africa. Praziquantel (PZQ) has been the only drug used to treat all schistosome infections because it is readily available, cost-effective, and has minimal side effects. Recent studies have shown that PZQ-resistant strains are emerging due to drug pressure. Other concerns are that PZQ does not kill the parasite during the reproduction stage, which is crucial because the disease directly results from eggs' entrapment in host tissue, thus increasing the individual's susceptibility to opportunistic infections. Therefore, it is critical to discover druggable targets and/or vaccine candidates for schistosomiasis. GAPDH is an enzyme found in the schistosome, which uses the NAD-binding domain component of its structure to generate energy motility and survival of the worm. Therefore, GAPDH is an important druggable target in the discovery and development of new anti-schistosomal agents. Therefore, the aim of this study is to recombinantly express and characterize the NAD-binding domain of GAPDH for future discovery, design, and development of new anti-schistosomal drugs. Competent JM109 bacteria cells were transformed with the NAD-binding domain of the GAPDH plasmid, followed by recombinant expression and affinity purification using a GST-Agarose column to obtain milligram quantities of the protein. Thereafter, biophysical characterization using FTIR and Raman spectroscopy was conducted

immediately after in silico analysis. Overall, the S. mansoni NAD binding domain of GAPDH was successfully characterized to provide a structural basis for the development of new anti-schistosomal drugs. Additionally, in silico analysis revealed Triosephosphate isomerase and Phosphoglycerate kinase as interacting partners, which may be critical in the discovery and design of small molecule inhibitors and the subsequent development of these as new anti-schistosomal compounds.

Molecular Biophysics / 89

Overexpression, purification, and characterization of the Hsp70.14 protein towards the discovery and development of new anti-cancer compounds

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Cancer remains one of the leading causes of death, with over 2 million cases worldwide. New therapeutic approaches are therefore under constant development, aimed at eliminating various pathways/mechanisms utilized by cancerous cells. Previous studies have investigated the molecular interaction between the Heat shock protein 70.14 (Hsp70.14) and the RING finger domain of Retinoblastoma binding protein 6 (RBBP6) to determine how this interaction contributes to the progression of cancer. Disruption of this interaction through the discovery and development of protein-protein interaction (PPI) modulators serves as one of the potential therapeutic approaches that can be used to reduce the development of cancer. Hence this present study aimed at the recombinant expression, purification, and characterization of the Hsp70.14 protein, one of the interacting partners of RBBP6. Hsp70.14 was expressed in competent Top10 E. coli cells, purified using affinity chromatography, and thereafter characterized using FTIR and Raman spectrometry. Additionally, in silico methods were used to computationally characterize and predict the structure of the protein. The results show that the protein predominantly contains hydrophilic residues, and its structure is made up of two alpha helices and three anti-parallel beta strands, which was successfully validated using a Ramachandran plot and a Qmean swiss model. FTIR results revealed a high number of carbonyl and hydroxyl groups present in the protein whilst Raman data displayed symmetric C-C stretching and CH2 twisting vibrations in the fingerprint region of the protein respectively. These characterizations provided the basis for the structural determination of the protein and the subsequent identification of the residues important in the interaction with this RING domain partner for the discovery and design of new anti-cancer biopharmaceuticals.

Quantum Biology / 90

Quantum optical mega-networks in biological architectures, and the computational capacity of life and the observable universe

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In this talk I will present an overview of our work analyzing mega-networks of tryptophan in biological architectures with numerical simulations and steady-state ultraviolet spectroscopy, providing opportunities for control of light-matter interactions in cellular organelles and neuronal bundles.

I will then, based on these insights and fundamental physical considerations, consider the computational limits of living systems and all matter in the observable universe. The implications for development of artificial intelligence(s) will also be discussed.

Networks of tryptophan – an aromatic amino acid with strong fluorescent response – are ubiquitous in biological systems, forming diverse architectures in transmembrane proteins, cytoskeletal filaments, sub-neuronal elements, photoreceptor complexes, virion capsids, and other cellular structures. We analyze the cooperative effects induced by ultraviolet (UV) excitation of several biologically relevant tryptophan mega-networks, thus giving insight into novel mechanisms for cellular signalling and control. Our theoretical analysis in the single-excitation manifold predicts the formation of strongly superradiant states due to collective interactions among organized arrangements of up to more than 100,000 tryptophan UV-excited transition dipoles in microtubule architectures, which leads to an enhancement of the fluorescence quantum yield that is confirmed by our experiments. We demonstrate the observed consequences of this superradiant behavior in the fluorescence quantum yield for hierarchically organized tubulin structures, which increases in different geometric regimes at thermal equilibrium before saturation – highlighting the effect's persistence in the presence of significant disorder. Our results motivate a revisiting of conventional assumptions about the computing limits of cytoskeletal and neuronal architectures, which are generally considered to signal via Hodgkin-Huxley action potentials (millisecond timescale). It is shown that these biosystems can harness superradiant effects (picosecond timescale) in tryptophan lattices to process orders of magnitude more information than exascale supercomputers, at significantly lower power consumptions, by operating extremely close to the Landauer bound for logically irreversible operations. The robustness of single-photon-excited superradiant states paired with subradiant states (second timescale) in biology thus offers a novel paradigm for understanding large collectives of quantum emitters and their quantum information processing limits in warm, wet, and wiggly environments.

Mathematical Biology / 91

Shear induced fractionalized dispersion during the Magnetic Drug Targeting in a permeable microvessel

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To predict the effective dispersion and saturated concentration of the drug carriers, a Caputo fractional
time derivative based dispersion model is generated. The impact of the memory effect dependence of solutions on previous instances on the shear augmented dispersion is analyzed during
the magnetic drug targeting in the microvessel. The magnetic nanoparticle are bound with the non-magnetic
materials/microgels with the therapeutic agents to prepare the drug carrier. A magnetic
field is created outside the body to control and accelerate the trajectories of the drug carriers. The nature of the blood flow into the vessel is considered as Casson fluid. The velocity of the drug carrier is solved analytically while the fractional-order dispersion equation is solved numerically by using the finite difference method. The influence of fractional-order parameter and model biological parameters such as rheological parameter, permeability parameter related to hydraulic conductivity, magnetization, volume fraction of nanoparticles, tumor-magnet distance, nanoparticle radius, drug elimination, and source term on the relative effective dispersion are discussed. The outcomes showed that both rheological parameters and volume fractions increase drug carrier particle concentration, and that saturating occurs at a later time as they increase. The higher magnetization, the permeability
parameter related to the hydraulic conductivity, and the source term, the faster drug-coated carriers are transported to the tumor site. In addition, we indicate that using small particle sizes, a high concentration of the drug-coated nanoparticles will be expected in the tumor area, and this slows the rate at which it reaches the saturation point.

Computational biophysics / 92

Probing binding affinity of human acetylcholine esterase for steroidal pregnanes as promising inhibitors through molecular modelling investigation

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Acetyl Choline Esterase (AChE) is one of the most important therapeutic targets for preventing and treating Alzheimer's disease. Studies have suggested the AChE inhibitory potential of pregnanes but the mechanism is still elusive. The aim of this study was to investigate the binding affinity of AChE enzyme for steroidal pregnanes in silico. Machine learning (ML) models were trained based on molecular fingerprints to rapidly screen a library of steroidal pregnanes retrieved from CHEMBL compound database for their half maximal inhibitory concentration (IC50) and inhibition constant (Ki) against AChE enzyme. Molecular docking, Molecular Dynamics (MD) simulation and MMGBSA free energy calculation were employed to further probe the binding affinity and decipher the binding interactions. Among 42 machine learning models assessed, Random Forest Regressor (RF) was a top model with high R-squared and low RMSE values. From 1,583 steroidal pregnanes, RF-based ML model screening revealed 843 pregnanes with pIC50 ≥ 5. Among these, 67 pregnanes with pKi ≥ 7 were suggested as promising AChE inhibitors. Atomistic simulations revealed 21-[(3-Hydroxy-2-naphthyl)oxy]pregnane-2-one (P1), 20-[2-(Imidazolidine-2-ylidene)hydrazono]pregnane-3beta-ol (P3) and 17-Hydroxy-3-oxo-19-nor-5beta,17alpha-pregnane-21-carboxylic acid, gamma-lactone (P4) as the Top Docking Pregnanes (TDPs). The top compound (P1) exhibited the best molecular contacts with the active site, interacting with the catalytic active site, peripheral anionic site (PAS), oxyanion hole and anionic sub-site through multiple hydrogen bonds and hydrophobic interactions. The AChE-TDP complexes exhibited structural stability and conformational flexibility in a dynamic environment. The RMSF plot revealed the interaction potentials of a loop around the PAS with TDPs. Also, P1 featured the strongest MMGBSA binding affinity (ΔG = -19.02±4.37 Kcal/mol) which was contributed mainly by key PAS residues. Furthermore, the TDPs were predicted to exhibit desirable drug-likeness, bioavailability and ability to cross the blood-brain barrier. Therefore, the in silico hits are suggested for experimental biophysical, biochemical and pre-clinical evaluation towards developing potent AChE inhibitors.

Biophotonics / 93

Using quantitative fluorescence correlation spectroscopy to study aggregation of the main photosynthetic antenna of plants

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Oxygenic organisms are capable of tuning their photosynthetic capacity on many different timescales to adapt to their environment. As such, they switch on photoprotective mechanisms to harvest solar energy with robust adaptability. Studying these mechanisms at the molecular level gives insight into how solar energy harvesting can sustain life on earth and which design principles can be used to improve man-made solar energy devices.

Photosynthetic antennae are pigment-protein complexes that absorb sunlight and transfer the energy towards reaction centres in photosystems. These complexes exhibit fast heat-dissipative processes that protect them from photo-induced chemical bleaching. In higher plants, aggregation of

these complexes amplifies heat dissipation, a process triggered by a lowering of the pH on the inner side of thylakoid membranes.

This presentation will describe a home-built fluorescence correlation spectroscopy setup and its use to study freely diffusing light-harvesting complexes of plants *in-vitro* at varying detergent concentration levels and pH levels. We will focus on the dynamics of the main plant light-harvesting complex, LHCII. Varying sizes of LHCII aggregates were investigated and their fluorescence lifetimes were obtained, allowing a direct comparison of aggregate sizes with quenching rates. In addition, we will show how triplet state lifetimes can be resolved through this technique on freely diffusing and immobilised single complexes.

Biophotonics / **94**

Real-time feedback-driven single-particle tracking spectroscopy of LHCII

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Single-molecule spectroscopy (SMS) has proven to be a powerful technique for investigating structure-function relationships in light-harvesting systems. In particular, SMS has unraveled dynamics in light-harvesting complexes that are hidden in ensemble measurements. However, the environment used in SMS experiments is a poor representation of the natural cellular environment, and therefore the results of these studies may be of limited physiological relevance. One limitation of conventional SMS experiments is the need to immobilize the complexes via surface attachment or to trap the complexes using, e.g., an anti-Brownian electrokinetic (ABEL) trap. This limitation is overcome by real-time feedback-driven single-particle tracking (RT-FD-SPT), a non-invasive technique that allows SMS measurements to be performed on single, freely diffusing particles for extended durations and with excellent spatiotemporal resolution. We studied different RT-FD-SPT methods using statistical analysis and simulations before using RT-FD-SPT to experimentally measure fluorescence lifetimes and emission spectra of single diffusing plant LHCII complexes. This paves the way for studies of the effect of surface immobilization as well as for studying single LHCII complexes in close-to-natural environments.

Computational biophysics / **95**

Molecular Dynamics Simulation on the Structural Stability and Solvation of Irinotecan in Water and Organic Solvents

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Irinotecan
Solubility
Solvation Free Energy

Computational biophysics / 96

DEVELOPMENT OF BIOCOMPATIBLE DRUG CARRIERS FOR IMPROVED DRUG LOADING AND RELEASE PROFILES

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Extensive research has focused on developing effective and biocompatible drugs and drug-delivery systems. Capsaicin, a natural compound found in hot peppers, has potential therapeutic properties, such as pain relief and antiinflammatory effects. However, its clinical application is limited by low cellular absorption, chemical instability, poor aqueous solubility, and some side effects, such as skin irritation and burning sensation. Lecithin, a phospholipid with biocompatibility and liposome-forming abilities, can be used in drug delivery systems. Both capsaicin and lecithin exhibit hydrophilic and hydrophobic characteristics, allowing them to self-assemble in aqueous solutions for drug loading and release.

Molecular docking and molecular dynamics, two crucial computational techniques in the fields of computational chemistry and structural biology, are instrumental for scrutinizing molecular interactions, especially in the context of drug discovery and protein-ligand interactions. In this study, we employ these methodologies to investigate the self-assembly behaviour of capsaicin and lecithin in an aqueous environment, revealing strong self-assembly into welldefined, arbitrarily shaped aggregates. The hydrophilic-hydrophobic nature of the materials enables improved drug loading and controlled release. Furthermore, the carrier enhances the physicochemical properties of capsaicin by forming stable complexes through nonbonded interactions. These findings inform the development of new drug delivery systems that utilize the self-assembly properties of amphiphilic molecules to improve the delivery and effectiveness of hydrophobic drugs.

The distance between the hydrophobic groups in capsaicin and lecithin appears to be smaller compared to the hydrophilic groups. The spacing ranges from 0.33 to 0.62 nm and 1.28 to 1.48 nm, respectively, this variation is because of an increased concentration of lecithin monomers, which ranges from 1-8. Increasing the concentration of lecithin has an impact on the rotation angle of capsaicin at the centre, reducing it from 123° to less than 60° and increasing the availability of water surrounding it. Additionally, an increase in lecithin concentration affects the arrangement of atoms attached to it. For example, the distance between the hydrogen of the hydroxyl group and the oxygen of the methoxy group increases from 0.25nm to 0.44nm, allowing more water to interact with capsaicin, thereby enhancing its ability to dissolve in water.

These observations suggest that hydrophobic groups play a crucial role in facilitating the rapid entrapment of capsaicin via hydrophobic forces. As the concentration of lecithin increases, the complex becomes more stable, strengthening the hydrophobic forces that hold capsaicin tightly and reducing its flexibility, which is crucial for effective loading and release.

Structural Biology / 97

The mechanisms of enzymes of the nitrilase superfamily

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Our goal is to determine the mechanisms of the nitrilase superfamily enzymes. These enzymes have common features such as their fold and conserved residues in their active sites (two glutamates, a lysine and a cysteine) but have a range of different activities. The superfamily derives its name from the nitrilases that convert nitriles to the corresponding carboxylic acids and ammonia, but most of the enzymes in the superfamily are amidases that convert amides to the corresponding carboxylic acid and ammonia. The reaction proceeds via the formation of a thioester intermediate. In our recent work [1] we identified the components of the active site that position the amide substrate for the attack by the cysteine on the carbonyl carbon of the amide. Our approach, which has led to several key insights, involves a combination of structure analysis, site directed mutagenesis, identification of intermediates by mass spectroscopy and quantum mechanical modelling [2]. An example of such an insight, shown in Fig. 1, locates the water molecule that is responsible for the hydrolysis of the thioester intermediate such that its lone pair overlaps with the LUMO of the carbonyl carbon.
Many amidase homo-oligomers, ranging from 2-8 monomers in different instances, have been crystallized, leading to extensive structural knowledge. The nitrilases, on the other hand, form spiral homo-oligomeric structures and, to date, none have crystallized in their complete, active form. The spiral structures are, however, amenable to structure determination by cryoEM [5]. In the case of Nit4 from Arabidopsis thaliana side chains in two adjacent monomers contribute to the active site pocket, playing an important role in substrate specificity. This enables enzymes to be tailored to a wide variety of substrates.

Computational biophysics / **98**

Investigation of the Impact of the Ionic Liquid on the Solubility of Acyclovir Derivative through Computational Analysis

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Acyclovir derivative is one of the most nucleoside analogs used as an antiviral drug for treatment of chickenpox, simplex virus infection and shingles. Acyclovir derivative like other analogs facing with poor solubility in water and organic solvent hindering its bioavailability and membrane permeation. To deal with this problem, Ionic liquid emerged to be potential candidate with the ability to improve the solubility of these drugs. To understand this, solvation mechanism was identified computationally. The findings shows that, Ionic liquid has high ability to improve the solubility of these drugs. From the results various factors that contribute to increase in the solubility of the drug was discussed including the contribution of van der waals and electrostatic interaction.

Computational biophysics / **99**

Investigation of the Structure, Stability, and Solubility of Psilocybin in Water and Pure Organic Solvents: A Molecular Simulation Study

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Background: Psilocybin, derived from magic mushrooms, has versatile medicinal potential, including neuroprotection and mental health benefits. FDA approval for clinical research suggests promise in treating anxiety, depression, and addiction. Nevertheless, clinical application is hindered by solubility issues and neurotoxicity. This study utilizes computational simulations to explore psilocybin's behavior in organic solvents, offering insights into its stability, structure, and solubility challenges, especially in contrast to its solubility in water.
Methodology: This study involves investigating psilocybin's characteristics in different solvents, including water and 35 common organic solvents. This is done through free energy calculations and detailed structural analysis. The solvation-free energy (ΔGsolv) is used to assess the interaction between psilocybin and the solvent, with a negative value indicating a preference for being in solution. The comparison between psilocybin forms A and B involves electronic structure calculations to establish their ideal gas reference states and interconversion energy. The research aims to relate these findings to the relative concentration of psilocybin forms in solution.
Results: The study validates the existence of two Psilocybin forms, A and B, with form B being thermodynamically more stable through free energy and DFT analysis. Hydrogen bonding significantly influences the solvation of Psilocybin form B, while aliphatic and non-hydrogen-containing solvents have minimal coulombic contributions. Alcohols and water exhibit different solvation behaviors, likely due to their unique properties. These results enhance our understanding of Psilocybin's stability and solvation in diverse solvent environments.
Conclusion: Findings suggest the thermodynamic stability of Psilocybin form B compared to A. Further studies are proposed to investigate different forms of solvents like ionic liquids
Keywords: Psilocybin; Solvents; Solubility; Stability; Free energy