DNA fragments, which is ideal for rapid detection using the MAMEF (Microwave-Accelerated Metal-Enhanced Fluorescence) platform[3], as well as diminishing the effects of DNases due to the elevated lysing temperatures.

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1576-Pos Board B527

Rapid Microbial Lysing and DNA Fragmentation by Microwave Focusing

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Bacterial infections are a major health problem worldwide. Identification of disease-causing organisms by culture-based approaches is time-consuming and often lacks sensitivity. Molecular approaches such as PCR and microwave-accelerated metal-enhanced fluorescence (MAMEF) assays1, are more sensitive and faster than traditional culture-based approaches, but require isolation of the target DNA. In order to determine the effect of both boiling and microwave irradiation on microbial lysing and DNA fragmentation, cultures of Neisseria gonorrhoeae and Listeria monocytogenes (108 CFU /mL) were either boiled (range 40° - 70°C) or lysed in a 900-watt microwave on isolator-mounted microscope slides, both with and without the assistance of disjointed antenna gold bow-tie structures. The temperatures of cultures were obtained prior to and after lysing and the resulting lysate cultured on selective agar plates. DNA isolation and fragmentation efficiency were determined by gel electrophoresis and PCR. N. gonorrhoeae lysed at a lower temperature (°C) than L. monocytogenes. Microbial lysing and DNA fragmentation was more effectively carried out in the presence disjointed gold triangle structures, but only when small sample volume were used. Standard boiling was successful for bacterial lysing and DNA fragmentation, but required higher temperatures and longer times than microwave focusing. PCR results suggest that low power microwave irradiation is ideal for PCR methods while higher microwave powers are required to generate DNA fragments ideal for MAMEF analysis. Microbial lysing and DNA fragmentation can be achieved by either boiling or microwave, but microwave lysing is more efficient for DNA fragmentation and is significantly faster. Microwave lysing is the recommended method when rapid isolation and DNA fragmentation is required.

1Melendez, et at. (2013). Blind Evaluation of the Microwave-Accelerated Metal-Enhanced Fluorescence Ultrarapid and Sensitive Chlamydia Trachomatis test by use of Clinical Samples, Journal of Clinical Microbiology, 51(9), 2913-2920.

1577-Pos Board B528

Palmitate Re-Directs Glucose Utilization in Type 2 Diabetic Hearts, Improving Function: A Metabolomic-Fluxomic Study

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Hyperglycemia and hyperlipidemia are two main traits of type-2 diabetes (T2DM). T2DM patients may develop a cardiomyopathy, and the excess in nutrients greatly contributes to systolic and diastolic dysfunction. The Randle cycle postulates that fatty acid (FA) utilization further impairs glucose utilization, impeding its oxidation. Yet recent evidence suggests that, when acutely infused, FAs such as palmitate (Palm) actually help in maintaining function in T2DM hearts stressed with high glucose and catecholamines. Thus, under conditions of sustained stress, lipids may be necessary to maintain function in stressed T2DM hearts. Using a novel procedure for translating metabolomics into metabolic fluxes, here we tested whether Palm is able to redirect the glucose fluxome in T2DM hearts, contributing to a better utilization/oxidation of glucose. We found that Palm, without inhibiting glycolysis, led to a 50% increase in glucose oxidation via the pentose phosphate [PP] pathway. Palm presence shifted the control of the glycolytic flux from phosphofructokinase to glucose uptake, glucose 6-phosphate dehydrogenase and glycogenolysis. Palm-induced remodeling of the glucose fluxome decreased the intracellular levels of glucose by 17-fold, owing to reduced uptake at maintained utilization. Moreover, it augmented the content of reduced GSH, via higher NADPH generation through the PP pathway. Our study provides a mechanistic explanation to the in vitro observation that FAs such as Palm are necessary for the T2DM hearts to maintain function when in presence of hyperglycemia and/or increased workload, by remodeling glucose utilization leading to a higher supply of reducing equivalents to the heart. Present findings suggest that in T2DM subjects the Randle cycle may apply to some but not all pathophysiological contexts.

1578-Pos Board B529

Modeling Host - Bacterial Biofilm Interactions in Lower Leg Chronic Wounds

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Chronic wounds are caused by a healing process often stalled at the inflammation, proliferation stages of repair. Each chronic wound displays its own dynamics in a complex 3-dimensional interplay of repair against a range of obstructing factors. These may include host and bacterial genetic makeup, medical and environmental conditions, counter-productive habits and the presence of an opportunistic poly-bacterial biofilm. Currently there is a rapidly growing need to create a treatment plan upon assessment of a wound followed by regular quantitative monitoring. Understanding the spatial and temporal variations in biochemical and biophysical wound parameters will aid a timely healing process. In-silico simulations can support these efforts by modeling the influence of the various factors. Examples are: proper use of smart wound dressings and prevention of the build-up of antibiotic tolerance and resistance.
We present results based on expansions of published models to simulate the destruction of wound bed collagen, the dynamic interplay of host neutrophils, macrophages, fibroblasts and keratinocytes against the detrimental effects of bacterial metalloproteinases (MMPs) under normal, low-oxygen or anaerobic conditions. These simulations also allow to assess the influence of a biofilm even though most of its interactions with host chronic wound-tissue are barely known or understood With growing knowledge computer modeling will aid in management of individual chronic wounds.

1579-Pos Board B530

Model for Aging and Cognitive Decline

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A population of neurons in the cerebral cortex of humans and other mammals organize themselves into vertical microcolumns perpendicular to the pial surface. Anatomical changes to these microcolumns have been correlated with neurological diseases and normal aging, and in particular in area 46 of the rhesus monkey brain the strength of microcolumns was shown to decrease with age. We have previously developed a model to simulate aging brains by constructing a microcolumnar network of neurons and allowing the neurons to undergo Brownian motion while being constrained by a harmonic force that weakens as a function of age. Now, we expand on this model by constructing and simulating the generated neural networks. By generating a young neural network from strong restorative forces, one can create an initial distant dependent connectivity. Then, we age these networks and presume that connectivity between neurons either weakens or severs as a function of neural displacement from initial neuronal positions. We aim to show that older networks are unable to efficiently shift between different firing regimes, providing a potential mechanism for loss of information processing in relation to microcolumnar structure.

Molecular Dynamics II

1580-Pos Board B531

Comparison of Activation Energy and Pore Dynamics in Liquid and Gel Phases of Electroporated Lipid Bilayers using Temperature Dependent MD Simulations

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The molecular level understanding of electroporation has been studied by few research groups [1, 2, 3] over the last decade. We have performed molecular dynamics simulation(MDS) of electroporation at different temperatures to find activation energy as well as pore dynamics in the gel and liquid phases of POPC and DPPC lipid bilayers.
The MDS of bilayers were performed using NAMD, the Particle mesh Ewald(PME) method, the all-atom CHARMM force field and an integrated time-step of 2 fs.
The bilayers were composed of 256 lipids which were solvated with TIP3 water molecules with a low KCl concentration. The MD simulations were performed in temperature range from 250 K to 350 K with varying electric fields (0.02 to 1 V/nm).
A plot of pore initiation rate as a function of inverse temperature showed Arrhenius type behaviour. The activation energy was determined to be 25.5 and

21.5 kJ/mol for the liquid phase of POPC and DPPC lipids respectively for an electric field of 0.3 V/nm, and reduces at higher fields. The activation energy in the gel phase of POPC increases to 28.8 kJ/mol at the same field. The pore closing time after the field is switched off was found to be longer in the gel phase than in the liquid phase. Remarkably, pores of radii ~0.7nm in the gel phase of POPC did not close even after 50ns, whereas they close completely within 10ns in the liquid phase.

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[2]PT Vernier, MJ Ziegler, Y Sun, WV Chang, MA Gundersen and DP Tieleman, J. Am. Chem. Soc., 128 (2006) 6288-6289.
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1581-Pos Board B532

The Carboxy Terminus of the Ligand Peptide Determines MHC Class I Complex Stability: A Combined Molecular Dynamics and Experimental Study

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Major Histocompatibility complex (MHC) class I proteins bind peptides of eight to ten amino acids to present them at the cell surface to cytotoxic T cells. The class I binding groove binds the peptide via hydrogen bonds with the peptide termini and via diverse interactions with the anchor residue side chains of the peptide. To elucidate which of these interactions is most important for the thermodynamic and kinetic stability of the peptide-bound state, we have combined molecular dynamics simulations and experimental approaches in an investigation of the conformational dynamics and binding parameters of class I molecules with optimal and truncated natural peptide epitopes. We show that the F pocket region dominates the conformational and thermodynamic properties of the binding groove, and that therefore the binding of the C terminus of the peptide to the F pocket region plays a crucial role in bringing about the peptide-bound state of MHC class I.

1582-Pos Board B533

Molecular Modelling in MRI Contrast Agents Interacting with Water Molecules: Hierarchical Clustering Method for Molecular Dynamics Data Analysis

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A revised method to compute mean residence time (MRT) from molecular dynamics (MD) simulations is reported. One of the most frequent scenarios in the modeling of biological related systems is to describe their interactions with the solvent that in most of the cases is water. This is important, for instance, in determining the three dimensional structure and function of proteins that will greatly depend on the affinity of certain sections of these biomolecules with water. The amount of interaction with water can be quantified by means of the so called MRT, which for this specific case is the average time that a water molecule stays within a certain distance (threshold) from a particular referential point of the model molecule. Determining this threshold and for how long it can be broken during a simulation and still counting as a single event is not a straightforward task. To calculate MRT from MD data our methodology uses hierarchical clustering analysis. We propose applying this methodology as a step for the computational design of novel contrast agents for Magnetic Resonance Imaging (MRI) taking into consideration that MRT of water interacting with a given contrast agent directly affects the quality of the MRI images. In addition, it is shown that the presented method is applicable in a wider range of scenarios in MD data analysis.

1583-Pos Board B534

His 95 Acts as a pH Gate in Aquaporin-4

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Aquaporins are trans-membrane channels that are responsible for the permeation of water across the cell boundary. Responding to environmental stresses such as osmolarity, voltage and pH is an important aspect of regulation of channel permeability. We demonstrate that aquaporin-4, a membrane water channel modulates water transport via pH sensing. Aquaporin-4 is expressed mostly on the cytoplasmic membrane of cells of the nervous system. It has been implicated in the formation of edema during stress caused to the brain and thus has medical relevance. In this work we combine a Molecular Dynamics based computational approach with empirical methods to identify the molecular mechanism involved in the regulation of the channel via pH . Using a Partial Least Squares (PLS) based machine learning algorithm, we perform Functional Mode Analysis (FMA) to elucidate collective motions in the protein that are responsible for opening and closing the channel pore. We find that the protonation of conserved histidine residue H95 opens the channel and locally increases the pore radius. We employ Essential Dynamics (ED) simulations to ascertain that the collective mode identified by the computational method can indeed switch the protein function on and off. This mechanism is then tested experimentally by expressing the protein on Xenopus oocytes. By controlling the pH on either side of the cell boundary the location of the pH sensor is identified with respect to the cell membrane. Finally using mutational analysis it is established that H95 is the residue responsible for the pH sensing.

1584-Pos Board B535

Structural and Dynamical Study of Bovine Carbonic Anhydrase II in the Presence of Substrate: An Essential Dynamics and Molecular Dynamics Simulation Study

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Activity, regulation and inhibition of Bovine Carbonic Anhydrase II (BCAII), like other enzymes, are associated with its conformational changes.Molecular dynamics simulation was performed for Free BCAII and BCAII in complex with Para-nitro phenyl acetate to investigate the effect of substrate binding on BCAII structure and dynamics. Each Simulation was done for 100ns, in water with GROMACS software package. DSSP and Essential Dynamics techniques were used for analyzing secondary structures and concentrated motions, respectively. Results of this study demonstrated that presence of Para-nitro phenyl acetate in BCAII active site increased RMSD in the secondary structure backbone resulting in increasing number of amino acids in alpha helix and beta sheet and as opposed to turns. Flexible regions are located in the N-terminal while, surface of the protein and its central domain and the c-terminal have low flexibility and high resistance against changes. Motions have been induced in protein surface and secondary structures. Reduced motion in several sites, including amino acids 52 to 58 and 173 to 175 was observed. Increased motion in several sites, including beta sheets and alpha helices was induced. Results are in good agreement with studies on BCAII knotted structure and resistance against conformational changes.

1585-Pos Board B536

Resolving the Mechanisms of Bacterial Resistance to Macrolide Antibiotics

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Macrolides are a class of commonly used antibiotics that target the bacterial ribosome and prevent protein synthesis in the affected cells. Ribosomal residues A2058 and G2505 in the protein exit channel are considered to be particularly important for macrolide binding. Unfortunately, due to extensive use of macrolides, bacterial resistance, caused by mutation or methylation of specific rRNA residues in the ribosome, has become a growing concern. How these changes in rRNA induce macrolide resistance on a molecular level is still unclear. Here, we investigated macrolide resistance using atomistic molecular dynamics simulations.

Presently, there are no force field parameters developed specifically for macrolides. Therefore, we have developed novel approaches for force field parametrization from first principles for large and bulky molecules, such as macrolides, using the Force Field Tool Kit plugin in VMD. Parameters were developed and validated for two commonly used macrolides: erythromycin and azithromycin. These macrolides were studied in wild-type and in two mutated ribosomes of E. coli: G2057A and A2058G. The simulation showed that both mutations caused rearrangements of the binding site and decreased hydrogen bonding between the macrolide and residue 2058. Surprisingly, the G2057A mutation prevented hydrogen binding to residue 2058 to a larger extent than the A2058G mutation.

1586-Pos Board B537

Molecular Modeling of Self-Assembly of Anticancer Drug Amphiphiles

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Recently, Drug amphiphiles (DAs) have been shown to form discrete and stable supramolecular nanostructures with high and quantitative drug loading1. A drug amphiphile consists of a hydrogen-bonding peptide sequence attached to a hydrophobic drug. Similar to peptide amphiphiles2, DAs also self-assemble into discrete and well-defined supramolecular structures. Using