the number of stress cycles is large enough. Fatigue is a major cause of failure in machinery; however there is no comprehensive theory of fatigue. To design machines, engineers use heuristic methods such as the Palmgren-Miner rule or S-N diagrams. Here, we investigate for the first time a molecular theory of fatigue failure. We aim to provide a theoretical basis for the heuristic methods engineers use to avoid fatigue failure and draw conclusions about the design of molecular machines such as kinesin or myosin motors.

3727-Pos Board B588

Mechanochemistry of Small Molecules: Into Which Bond does the Force Go?

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Regulation of (Bio)chemical reactions by mechanical force has been proposed to be fundamental to cellular functions[1]. Atomic force microscopy and molecular force probe experiments suggested an enhancement on the reactivity of thiol/disulfide exchange[2] and ring-opening of cyclobutene[3], respectively. Recently, we have performed hybrid quantum mechanical molecular mechanical simulations in combination with transition path sampling on the thiol/disulfide exchange. We could show that stretching a molecule can significantly shift the transition state, and also affects degrees of freedoms other than sole bond stretching [4].

In order to understand into which degrees of freedom the force goes, we have developed a force distribution analysis method for ab initio simulations, a simple scheme to deduce pairwise forces from non-pairwise quantum mechanical descriptions, which is transferable to any other (bio)chemical molecule for which internal stresses are of interest. The application to the ring-opening of cyclobutene shows how mechanical stretching forces propagate into the bonds of the reactive system, leading to both compressive and tensile forces in the strained cyclobutene. The force distribution allows to directly relate internal forces in bonds to mechanochemical events of bond scission.

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3728-Pos Board B589

QM/MM Molecular Dynamics Methods Applied to Investigate Cellulose Fibers Hydration

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The imminent lack of fuel instigated by the upcoming end of the world's oil reserves has increased the interest in new energy sources. Biofuels are an alternative largely used in Brazil, where gasoline and ethanol consumption are about the same. However, to the ethanol production, just the sugarcane juice is employed and a large amount of biomass is not efficiently used. This colossal amount of resources, specially the cellulose fibers, is the basis to the production of the so called second generation biofuels.

Nevertheless, the crystalline structure of the cellulose fibers is a big challenge, since enzymes do not efficiently degrade this kind of structure, however they do efficiently break down imperfect fibers. Structural studies had shown the importance of a hydrogen bond web to stabilize these fibers. In this work, we are studying the behavior of this fiber using molecular modeling tools, in order to develop a technic to break down these hydrogen bonds, which should lead to the production of single chains or even a less structured fiber.

In this work, QM/MM MD simulations of a fully hydrated cellulose fiber segment were carried out to observe the influence of the hydration in the fiber stability. The structure obtained using these simulations were used as input in a DFT study of a small portion of the fiber. These QM simulations were carried out to study the energy and frequency of the inter-chain hydrogen bond. Our simulations are showing a very stable frequency value to these bonds and we could use it to break these H-bonds using QM/MM calculations, to produce a resonance phenomenon.

3729-Pos Board B590

Structural Thermodynamics of NMDA Receptor Ligand-Binding Domains

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Neuronal signaling is based on the release and detection of glutamate within the synaptic cleft. Regulation of this signaling has implications in both learning and memory, while dysfunction is implicated in a variety of neurological disorders, including Parkinson's and Huntington's diseases. Glutamate in the synaptic cleft is detected by glutamate receptors (GluRs), which are ligand-gated ion channels. N-methyl-D-aspartate receptors (NMDARs) are a class of GluRs that require the binding of both glycine and glutamate for receptor activation. Here, the free energy landscapes governing large-scale conformational transitions in the isolated ligand-binding domains (LDBs) of the NMDAR subunits NR1, NR2A, and NR3A are computed for both apo and holo forms using umbrella sampling simulations. The effects of both agonist insertion and amino acid substitutions on conformational stabilities in the various LBDs are examined.

3730-Pos Board B591

Ligand-Protein Interaction Studied by Computer Simulation and Time-Resolved X-Ray Crystallography

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Recently, high resolution x-ray crystallography demonstrated breathing motion of internal cavities in concert with ligand migration in myoglobin(Mb) [1]. Continuous pulsed illumination of carbomonoxy-Mb crystals at low temperatures has illustrated structural changes around each cavity in response to ligand migration. In the present study, we examined the effect of the breathing motion on the potential of mean force(PMF) for ligand-protein interactions by using molecular dynamics simulation and experimental derived from the x-ray study[1]. Conformational sampling of Mb was performed by NPT molecular dynamics simulation for 92 ns. We introduced three-dimensional lattice of regularly spaced grid points, and evaluated PMF at each point by the implicit ligand sampling method [2]. The effect of the breathing motion of Mb on the ligand-protein interaction was illustrated by the difference map of PMFs for Mb structures before and after light illumination. Our results show ligand escaping mechanism via Xe1 pocket and gate opening between Xe2 pocket and Xe3 pocket.

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3731-Pos Board B592

Molecular Dynamics Studies of Protein Targets for Cancer

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In this meeting, we present the molecular dynamics studies of two protein targets for cancers. The one is epiregulin (EPR), which is a member of the epidermal growth factor family and a factor affecting pancreatic cancer. Recently, it was observed that the binding affinity of EPR without S-S bonds for an antibody is lower than that of EPR with S-S bonds, and that the affinity of a long form type (extracellular domain) of EPR without S-S bonds is higher than that of a mature type without S-S bonds. To investigate the effect of the S-S bonds on the structural stability of EPR, we have performed molecular dynamics (MD) simulation for these three types of EPR. From our simulations, it has found that the S-S bonds reduce the structural fluctuation of EPR, and the structure of the mature domain in the long form type is similar to the mature type with S-S bonds. The other protein target is the loop region between fibronectin type III domains of roundabout-1 that is a receptor for Slit1 and Slit2 in axon growth cones and differentially expressed in cancers. We have investigated the structural stability and thermodynamic property of the loop region using MD simulation. We have calculated two types of peptide of different size: the peptide composed of 26 amino acid residues corresponding to the full region of the fluctuating loop, and the shorter one of 20 amino acid residues. In the 20 amino acid peptide calculations, we have observed two different stable conformations, which are stable during more than 1.5 μs. We have found that for the one conformation, the intramolecular interaction energy contributes to the thermodynamical stability, while for the other one, water binding to the turn part of the peptide is dominant contribution.

3732-Pos Board B593

Accelerated Molecular Dynamics Simulations of Thrombin-Thrombomodulin Reveal Potential for Entropic Allostery

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The specificity of the serine protease thrombin is altered from procoagulative cleavage of fibrinogen to anticoagulative cleavage of protein C upon distal binding of cofactor thrombomodulin (TM). The fourth EGF-like domain of TM (TM4) is necessary to elicit this response, though it makes no direct contact with thrombin. While this effect can be described as allosteric, crystal structures of apo and TM bound thrombin do not reveal a significant structural change, making a significant enthalpic contribution to allostery unlikely. We

hypothesize that allostery in thrombin may instead occur through a primarily entropic mechanism.
To investigate the potential for entropic allostery in thrombin a series of accelerated molecular dynamics (AMD) simulations were performed. AMD applies a bias energy to the underlying potential of a classic MD system to model longer timescales on which allosteric events are more likely to occur. Residue by residue cross-correlation analysis and community network models constructed from the resulting trajectories are indicative of an allosteric pathway between the thrombin active site and TM4. In addition, order parameters back-calculated from trajectories of apo and TM bound thrombin, show differential dynamics near the thrombin active site and TM binding site. Together these results show that TM binding alters thrombin dynamics in a manner that could reasonably contribute to the observed change in specificity through entropic allostery.

3733-Pos Board B594
Computational Studies of a pH Responsive Histidine-Modified Cardiac Troponin I
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Cardiac troponin I (cTnI) functions as the molecular switch of the thin filament. Studies have shown that a A164H button engineered into cTnI enhances inotropic function under pathophysiological challenges. In vitro studies of myofilament calcium sensitivity and sarcomere shortening kinetics in intact and permeablized myocytes at baseline (pH 7.4) indicated similar cellular contractile function and myofilament calcium sensitivity between myocytes expressing wildtype cTnI and cTnI A164H while A164R showed a hypercontractile phenotype associated with increased myofilament calcium sensitivity. Under acidic conditions, compared to depressed function in myocytes with wildtype cTnI, myocytes expressing cTnI A164H maintained myofilament calcium sensitivity and contractile performance comparable to the calcium sensitizer cTnI A164R. The role of histidine modified cTnI was assessed by molecular dynamics (MD) simulations and pKa calculations of the wildtype and histidine or arginine-modified cTnI (148-173): cTnC (1-90) complex. The simulations showed similar conformations between the wildtype and the deprotonated cTnI A164H variant. In contrast, simulations of protonated cTnI A164H and cTnI A164R showed diverse conformation changes, both of which included the formation of a cTnI His 164 and cTnC Glu 19 salt bridge. pKa calculations showed no significant pKa shift for all ionizable residues except for cTnI His 164 and cTnC Glu 19 when the salt bridge is formed. The data shed light into the potential mechanism of pH activation of cTnI A164H and the importance of electrostatic interactions in governing the biophysical adjustments in troponin function necessary for nuanced modulation of myofilament function in response to changes in the cytosolic milieu.

3734-Pos Board B595
Pressure-Induced Denaturation of Proteins Studied by Generalized-Ensemble Molecular Simulations
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The pressure dependence of several proteins has recently been studied both experimentally and theoretically. Some of the experiments showed that proteins are denatured under high pressure conditions. We would like to understand the molecular mechanism of the pressure-induced denaturation of proteins by using molecular dynamics simulations.
Molecular dynamics simulations have been widely used for studying biomolecular systems. However, the molecular simulations of biomolecular systems often get trapped in local minimum energy states. One way to overcome such a difficulty is to use generalized-ensemble algorithms. Using generalized-ensemble algorithms in molecular simulations, we can sample protein conformations efficiently and calculate physical quantities accurately. We have recently developed a generalized-ensemble algorithm for the isobaric-isothermal ensemble. This method can be used to calculate accurate temperature and pressure dependence of biomolecular systems.
In this study we performed molecular simulations of ubiquitin, which is denatured under high pressure conditions. We performed generalized-ensemble molecular dynamics simulations by the NAMD program package. In these simulations, we used one temperature value, 300 K, and one hundred pressure values in range from 1 bar to 10,000 bar.
We calculated the fluctuations of the distance between all pairs of amino acid residues. A large distance fluctuation of an amino acid pair means that increasing and decreasing pressure makes the amino acid residues move largely and therefore it is possible that local protein structure changes are induced with increasing and decreasing pressure. The amino acid residues which were largely displaced under high pressure conditions in the experiments correspond to the largely fluctuated amino acid residues in the molecular simulations.

3735-Pos Board B596
An Elastic Network Coarse Grained MD Model Tested on Protein with Hinge Movement upon Ligand Binding
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In recent years, coarse grained (CG) models, with a resolution at the residue level for proteins,[1,2] have gained great popularity, due to its balance between accessible time scale and detail level. However, because of the coarse description of the residues, electrostatic interactions arising from the electron distribution within the coarse grained beads are lost, and the model fails to consistently reproduce protein secondary and tertiary structure. Therefore additional restraints stabilizing the initial structure are imposed, allowing the CG model to maintain the correct structural scaffold,[3] but impairing the ability to study protein conformational changes.
Here I will present a study comparing the capability of selected CG models to describe the conformational change of a two-domain protein that undergoes fully closure upon ligand binding. With the proposed domELNEDIN model, where an elastic network is set up only internally in the protein domains, we are able to observe the expected conformational change. To also improve the description of the protein-ligand interaction in the CG model, representations of the ligand using bead types similar to those introduced recently for polarizable CG water[4] were tested.
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3736-Pos Board B597
Slow Dynamics in Protein Fluctuations Revealed by Time-Structure Based Independent Component Analysis
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Protein fluctuations in equilibrium are an important factor for its structural change and function. It has been found that a small number of low-frequency modes in a protein, determined by normal model analysis or principal component analysis, account for its large fluctuations and contribute to its structural change significantly. However, dynamical aspects of protein fluctuations and their roles in protein function remain unclear. Recently, we have proposed the time-structure based independent component analysis (tICA) to reveal slow dynamics of a protein [Y. Naritomi and S. Fuchigami, J. Chem. Phys. 134, 065101 (2011)]. In this paper, we focused on domain motions of the target protein by using rigid-body domain analysis. However, a protein shows not only domain motions but also a variety of motions of main- and side-chains. In the present study, we investigated protein main-chain dynamics on a long time scale by using all-atom molecular dynamics (MD) simulation and the tICA. As a target protein, we selected lysine-, arginine-, ornithine-binding protein (LAO), which undergoes a large structural change upon ligand binding. A one-microsecond MD simulation of apo-LAO in explicit water was performed using MARBLE and the CHARMM22/CMAP force field parameters. Applying the tICA to the simulation result yielded slow modes of the LAO, which represented both domain and local motions. Other analyses confirmed that these motions were actually occurred on the expected slow time scale, suggesting that the tICA is powerful for analyzing slow dynamics of proteins.

3737-Pos Board B598
Modulation Mechanism on the Conformational Diversities of Biantennary Complex-Type N-Glycans in Water
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The core fucosylation and the introduction of bisecting GlcNAc of N-glycans are known to modulate their conformations and considered as one of the fundamental means for regulating their binding affinities to lectins. In this presentation, we show details of the modulation mechanism, which was not fully elucidated in the past, by conducting replica-exchange molecular dynamics simulations of four distinct N-glycan molecules (Bi9, BiB10, BiF10 and BiBF11). The global conformation was highly correlated with the motion of 1-6 arm. The N-glycan without core fucosylation and the bisecting GlcNAc shows highest flexibility