electrophoresis. The band with intact HSA was cut out and the remainder of the gel was cut into four different pieces. Gel sections then were digested with trypsin. Levels of MSO in the resulting peptides were assessed by LC-MS/MS and data analysis was performed using the Skyline software package.
Results: A group comparison between non-smokers (control) and smokers showed a slight increase in the levels of MSO found in intact HSA of smokers relative to non-smokers. Regions of gels with proteins of lower mass than intact HSA showed that degraded fragments of HSA in urine of both smokers and non-smokers have higher levels of MSO than are found in intact HSA.
Conclusions: HSA in smokers has statistically significant higher levels of MSO than HSA in non-smokers. However, the higher levels of oxidation in smokers are concentrated in partially degraded HSA. At the moment it is not possible to say unequivocally whether oxidized HSA is more likely to be cleaved and cleared, if cleaved protein is more likely to be oxidized before clearance, or both.

246-Pos Board B11
Dynamics and Energetics of Elongation Factor SelB in the Ternary Complex and the Ribosome
Lars V. Bock[1], Niels Fischer[2], Holger Stark[2], Holger Stark[2], Helmut Grubmüller[1].
[1]Theoretical and Computational Biophysics, MPI for biophysical chemistry, Goettingen, Germany, [2]Structural Dynamics, MPI for biophysical chemistry, Goettingen, Germany.
SelB is an elongation factor specialized to deliver the selenocysteine (Sec) tRNA to the ribosome by recoding the UGA stop codon on the mRNA. Initially the tRNA is in complex with selB and GTP forming the ternary complex (TC). High-resolution cryo-EM structures of intermediates of the Sec incorporation pathway uncover large-scale conformational changes of the ribosome and the TC. To complement the structural information with energetics and rapid dynamics, we performed extensive all-atom molecular dynamics simulations of the ribosome with bound TC as well as of the free TC in solution. The simulations of the free TC were started after extracting the TC from the ribosome-bound cryo-EM structures. The TC was found to rapidly interconvert between the different conformations allowing us to construct the free-energy landscape of the involved motions. This free-energy landscape indicates that the intrinsic large-scale conformational changes of the tRNA and SelB during the delivery to the ribosome are not rate-limiting to the process. In simulations of the free TC started from the GTPase-activated ribosome-bound conformation, the TC rapidly transitions into an inactivated conformation, showing that the GTPase-activated state is strongly stabilized by the ribosome. The simulations of the full ribosome with bound TC in the intermediate states allow us to identify the motions that are rate-limiting to the process of tRNA delivery and to identify the molecular mechanism of the domain closure of small ribosomal subunit upon tRNA decoding.

247-Pos Board B12
Polyethylene Glycol Conjugation Enhances Mosquito-Larvicidal Activity of *Lysinibacillus Sphaericus* BinA Protein
Mahima Sharma[1,2], Ramesh S. Hire[2], Ashok B. Hadapad[2], Gagan Deep Gupta[2], Vinay Kumar[2,3].
[1]Life science, Homi Bhabha National Institute (HBNI), Mumbai, India, [2]Bhabha Atomic Research Centre, Mumbai, India, [3]Homi Bhabha National Institute (HBNI), Mumbai, India.
Mosquitoes are known to spread human diseases like West Nile fever, dengue, malaria, zika etc. accounting for millions of deaths worldwide. *Lysinibacillus sphaericus*, a gram positive, spore producing commensal soil bacterium, has been used worldwide for controlling mosquito population like Culex and Anopheles, and is regarded as safe against non-target organisms. Binary toxin, composed of BinA (41.9 kDa) and BinB (51.4 kDa) component proteins, is responsible for the high larvicidal activity of several *L. sphaericus* strains. The two proteins exert high toxicity when administered together. BinA alone displays larvicidal activity, in the absence of BinB, *albeit* at reduced levels. But instability, shorter half-lives and rapid proteolytic digestion can limit their use as an effective insecticide. We for the first time demonstrate the beneficial effect of PEGylation (covalent attachment of polyethylene glycol) on mosquito-larvicidal activity of BinA protein. PEG-protein conjugates were synthesized using PEG-isocyanate polymer. The resulting bio-conjugates were purified to homogeneity by column chromatography methods. These were characterized by various biophysical methods like MALDI-TOF, DLS, DSF and CD. Two different isoforms of PEG-BinA conjugates are expected from biophysical analysis, which appear to be mono-PEGylated but may differ in the site of PEG attachment to BinA protein. The PEGylated proteins displayed preservation of protein's native structure and exhibited improved thermal stability by about 3-5 ^{o}C. The PEGylated proteins were also checked for stability against complex proteolytic environment. Regardless of the site of modification, the two isoforms showed a remarkable 7-fold improvement in the larvicidal activity of BinA protein against 3rd instar Culex larvae, compared to the unmodified protein. The PEGylation of recombinantly produced BinA can be achieved easily. It promises a judicious approach towards mosquito population control.

248-Pos Board B13
A Novel Electrostatic Regulatory Mechanism in a Calcium Binding Protein, L-plastin
Jonn Keenan Fanning, **Van A. Ngo**, Hiroaki Ishida, Hans Vogel, Sergei Yu. Noskov.
Biological Sciences, University of Calgary, Calgary, AB, Canada.
Plastins are a group of highly conserved actin binding proteins. L-plastin is a human isoform of this protein found active in hemopoietic cells. This isoform is also expressed in cancer cells. The N-terminal of this isoform contains a Calcium-binding EF-hand domain that allosterically regulates this protein for binding to its target. It is, however, very difficult to delineate the effects that underlie the allosteric mechanism. To investigate the regulatory mechanisms of the activation of this protein domain, we have used both computational and experimental methods. Working with calcium in MD simulations has previously caused significant problems as classical force fields are not well equipped to deal with calcium. This is because calcium is a divalent ion, which can induce a strong local electrostatic field, and is capable of charge transfer. Although classical force fields are insufficient, a new polarizable force field now known as a Drude force field, provides a more accurate solution to this problem. We describe results from simulations with both Classical and Drude force fields for the calcium-sensitive regions of L-plastin. These ongoing simulations have already provided valuable insight an unexpected and previously unseen electrostatic regulatory mechanism of L-plastin. In addition to computational simulations, to further validate this novel mechanism we obtained promising experimental validation using a number of biophysical methods including isothermal calorimetry (ITC) and differential scanning calorimetry (DSC). Although we are looking into this mechanism in L-plastin, the conserved nature of this protein may indicate that this mechanism is present in a wide range of related proteins, and may help provide us with a deeper understanding of the mechanisms associated with calcium activation and deactivation.

249-Pos Board B14
Structural Destabilization of Tropomyosin Induced by a Cardiomyopathy-Linked Mutation
Thu N. Ly[1], Inna Krieger[2], Young-Ho Yoon[3], Dmitri Tolkatchev[1], Fadel A. Samatey[3], Alla S. Kostyukova[1].
[1]Chemical Engineering and Bioengineering, Washington State University, Pullman, WA, USA, [2]Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX, USA, [3]Trans-Membrane Trafficking Unit, Okinawa Institute of Science and Technology, Okinawa, Japan.
In striated muscle, thin filaments, composed of F-actin, and thick filaments constitute the basic contractile units called sarcomeres. Tropomyosin (Tpm), a two-stranded coiled-coil protein, binds along the actin filaments through head-to-tail polymerization, protects and stabilizes the thin filaments. The N-terminus of Tpm orients toward the pointed end of thin filament, where it interacts with pointed end-binding proteins such as tropomodulin (Tmod) and leiomodin (Lmod) to maintain the uniform filament length critical for proper sarcomeric functions. Recently, a hypertrophic cardiomyopathy-associated mutation, R21H, has been identified in striated muscle Tpm (Tpm1.1) with molecular mechanism of perturbing muscular function unknown. We designed, expressed, and purified the Tpm chimeric peptide αTM1a$_{1\text{-}28}$Zip. The peptide consists of 28 N-terminal residues of Tpm1.1 followed by 18 C-terminal residues of the GCN4 leucine zipper domain. The peptide was crystallized and its structure was solved. To study how this mutation affects Tpm1.1, we introduced the mutation R21H in the peptide. An effect of the mutation was studied *in silico* using molecular dynamics simulation (MDS) and *in vitro* by circular dichroism (CD). Temperature measurements using CD were conducted to characterize the effect of the mutation R21H on thermal stability of the αTM1a$_{1\text{-}28}$Zip peptide alone and its complexes with Tmod and Lmod fragments containing Tpm-binding sites. CD data showed that the mutation R21H caused a significant decrease in the helical content and structural stability of αTM1a$_{1\text{-}28}$Zip. Complexes formed between the αTM1a$_{1\text{-}28}$Zip[R21H] peptide and Tmod or Lmod fragments were less stable than those formed with wild-type αTM1a$_{1\text{-}28}$Zip. All CD data were in agreement with MDS results which showed that the mutation R21H significantly altered the coiled-coil structure of αTM1a$_{1\text{-}28}$Zip. We suggest that the mutation R21H destabilizes Tpm structure by disrupting local salt bridges formed between residues Arg21 and Glu26 on opposite strands.