for studies of purified in vitro studies of DNA mismatch interactions from Thermus Aquaticus MutS and MutL. In this poster we present our recent progress developing single molecule FRET assays for more complex studies of DNA repair. In particular, we highlight our work using unnatural amino acid engineering and other methods for site-specific labeling of yeast repair proteins, detailed analysis of kinetics to reveal nucleotide regulation of protein conformations, and steps toward studies in live cells.

353-Pos Board B133

Cardioprotective Effect of Exercise Training in Heart Failure Rats: Exercise Training Reduces Oxidative Stress Induced Nuclear Genomic Fragmentation

Karin Solvang-Garten[1], Morteza Esmaeili[1], Tone Bathen[1], Morten Høydal[1], Muhammad Shakil Ahmed[2], Håvard Attramdal[2], Øyvind Ellingsen[1], Tomas Stølen[1].
[1]Norwegian University of Science and Technology, Trondheim, Norway, [2]Oslo University Hospital, Rikshospitalet, Oslo, Norway.

Alterations in energy metabolism are hallmarks of heart failure (HF) following myocardial infarction (MI). The failing heart is commonly described as an energy-starved engine that has run out of fuel, and myocardial energetics is therefore a topic of considerable interest. Markers of oxidative stress are elevated in HF and have been correlated with myocardial dysfunction. Increased oxidative stress can induce DNA damage, and the main repair mechanism of oxidative DNA damage is Base excision repair (BER) and Single strand break repair (SSBR). Defects in SSBR in post-mitotic cells have been associated with human diseases. Exercise training is an effective treatment for HF. Therefore we aimed to investigate the effect of exercise training on the nuclear genomic integrity in HF rats.

MI was induced in 30 female Sprague Dawley rats by surgical ligation of the descending coronary artery. HF was present 4 weeks after ligation. HF rats were then randomized to remain sedentary (n=10) or exposed to either moderate (n=10) or high intensity (n=10) aerobic interval training. Sham sedentary rats (n=10) acted as controls. After 6 weeks of exercise training, H2O2 induced genomic DNA fragmentation was studied in isolated left ventricular cardiomyocytes through an alkaline single cell gel electrophoresis assay. Cardiomyocytes from HF sedentary rats had an increase in H2O2 induced genomic fragmentation by 29% relative to sham, exercise training reduced the genomic fragmentation towards sham levels. 31P NMR experiments revealed reduced PCr concentration in HF and exercise training did not change the PCr levels. However, both moderate- and high intensity training resulted in an increase in cardiac ATP concentration in HF rats. Exercise training seems to induce a cardioprotective effect to increased oxidative stress in HF rats and possibly affects BER/SSBR pathways.

354-Pos Board B134

Enhanced Dynamics of Mismatched Base Pairs Associated with Msh2-Msh6 Recognition

Yan Li, Manju Hingorani, Ishita Mukerji.
MB&B, Wesleyan University, Middletown, CT, USA.

The DNA mismatch repair (MMR) system guards the integrity of genetic material by scanning and correcting errors in a post-replicative manner. In eukaryotic cells, the initiation of MMR is achieved by recognition by MutS homologs (Msh). The Msh2-Msh6 heterodimer plays this important initial role in recognizing single base mismatches and small insertion/deletion loops (IDL) in MMR. Although Msh2-Msh6 recognizes mismatched DNA with high affinity, the exact mechanism by which Msh2-Msh6 distinguishes different types of mismatched base pairs from a large excess of canonical Watson-Crick base pairs is still unknown. In this study, we use the intrinsic fluorescent probe 6-methylisoxanthopterin (6-MI, guanosine analog) in the context of the ATFAA (F = 6-MI) pentamer sequence where it exhibits enhanced fluorescence, to measure the binding affinity of S. cerevisiae Msh2-Msh6 to different single base pair mismatches. Fluorescence anisotropy measurements reveal the following order for Msh2-Msh6 mismatch bp binding affinity: A:A ≈ A:G > A:C ≈ G:T ≈ +T > T:T ≈ G:G > T:C ≈ G:C. Fluorescence intensity measurements suggest a greater degree of DNA distortion accompanies binding to well-recognized mismatches. We employ Förster resonance energy transfer to measure the bending angle and compare with that observed in MutS/Msh2-Msh6 co-crystal structures. We have also investigated DNA dynamics upon Msh2-Msh6 binding using time-resolved fluorescence spectroscopy. Specific placement of the probe at the mismatch site or adjacent to it reveals significant local motion prior to protein binding. We observe that high affinity binding is associated with those mismatches that exhibit the greatest amount of motion. Protein binding stabilizes mismatch local motion, which is consistent with Phe intercalation at the site, as observed in Msh2-Msh6-DNA co-crystal structures.

355-Pos Board B135

Resolving the Kinetic States of a Proofreading DNA Polymerase

Szu-Ning Lin[1], Jordi Cabanas Danes[1], Tjalle P. Hoekstra[1], Douwe Kamsma[1], Gerrit Sitters[1], Martin Depken[2], Remus T. Dame[3], Gijs Wuite[1].
[1]Physics and Astronomy, Amsterdam, Netherlands, [2]Bionanoscience, Applied Sciences, Delft, Netherlands, [3]Chemistry, Leiden, Netherlands.

DNA polymerase (DNAp) carries out DNA replication. To ensure high fidelity in replication it is selective in incorporating correct nucleotides and in correcting mismatches. Using single molecule tools we have investigated the kinetic states of DNAp molecules and have proposed a kinetic model describing the interplay between replication and proofreading of DNA polymerase [Tjalle P. Hoekstra, 2014]. Based on new data we have refined the previous kinetics models of T7 DNAp and have found evidence for the existence of previously unidentified pausing states.

The model predicts that the short pauses are related to DNAp concentration and that exonucleolysis activity of DNAp is force independent. To test the validity of our model, we investigated the impact of low DNAp concentration (less than 10 nM) and low force (less than 6 pN).

Our new results indicate as predicted that the duration of short pauses increases when DNAp concentration is reduced. Interestingly we also find the existence of a previously unobserved very long paused state. The nature of this pause state still needs to be determined. We characterized the behavior of DNAp at low force in a parallelized manner using a newly developed Acoustic Force Spectroscopy (AFS) method. These experiments reveal that exonucleolysis events indeed occur at low force as predicted by our model. The confirmation of both predictions validates our model and sheds further insight into the detailed kinetics of DNAp.

356-Pos Board B136

Binding Dynamics of the Holliday Junction with Yeast MutS Homolog Msh4-Msh5

Sudipta Lahiri, Manju Hingorani, Ishita Mukerji.
Molecular Biology & Biochemistry, Wesleyan University, MIddletown, CT, USA.

MutS homologs (Msh) are conserved in all organisms and they are involved in the process of mismatch recognition and repair. In *Saccharomyces cerevisiae* there are five MutS Homologs (Msh2 through Msh6) that participate in repair and recombination processes. The heterodimeric protein complexes, Msh2-Msh3 and Msh2-Msh6, play a role in mismatch repair. MutS homolog Msh4 and Msh5 are not involved in mismatch recognition and repair and genetic studies in *S. cerevisiae* and mouse indicate that Msh4-Msh5 are responsible for gamete viability during meiosis, recombination and chromosomal segregation. Recent studies have shown that human Msh4-Msh5 recognizes and stabilizes Holliday or four-way DNA Junctions (4WJ), an intermediate in double stranded break repair. Our efforts are focused on elucidating structure-function relationships of *S. cerevisiae* MutS homolog Msh4-Msh5 by specifically studying the binding interaction of Msh4-Msh5 with DNA 4WJs. We have successfully expressed and purified *S. cerevisiae* Msh4-Msh5 in *E. coli* cells as both a heterodimeric protein complex as well as Msh4 and Msh5 monomers. Our initial data demonstrates that the purified proteins are active and binds with high affinity to DNA 4WJ. The binding affinity is in the nanomolar range and similar to that previously reported for human Msh4-Msh5. To study the effect of Msh4-Msh5 on junction conformation, we are using Förster resonance energy transfer. These measurements also probe whether Msh4-Msh5 preferentially binds to the open or stacked form of the junction. These measurements will also address whether stabilization of the stacked conformation is functionally related to suppression of recombination.

Protein-Nucleic Acid Interactions I

357-Pos Board B137

Elucidating the Transition Dynamics of HIV-1 Reverse Transcriptase using Single Molecule FRET

Mahipal Ganji, Elio Abbondanzieri.
Bionanoscience, TU Delft, Delft, Netherlands.

The reverse transcriptase (RT) of HIV has been a focus of research owing to its central role during the infection of a host cell. Although, currently there are thirteen antiretroviral drugs targeting RT in clinical therapy of HIV AIDS, HIV has developed resistance to these drugs. Understanding the mechanism of inhibition of RT's function and its interaction with nucleic acids will aid in discovering improved viral therapies. Previously, we developed an smFRET assay to study RT-nucleic acid interactions and showed that RT dynamically