Scientific Meeting
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AUSTRALIAN SOCIETY FOR BIOPHYSICS

Abstract: 172P

Investigating molecular dynamics and sequence dependency of invading DNA strand in Homologous recombination double strand break repair

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DNA double strand breaks (DSBs) occur daily in each replicating human cell. The small number (~5-50) unavoidably generated during cellular replication can pose a high risk due to the fact they involve lesions on both DNA strands, negating the built-in redundancy of double stranded DNA. Increased cellular stress, like that from exogenous agents such as smoke and alcohol, or via genetic deficiencies, can result in increased DSBs as well as delays and deficient repair processes. A small increase in the DSB events due to such factors are key mechanisms underlying multiple human diseases, including various cancers and neurodegeneration. Investigation of these rare DSB and repair events is challenging due to limitations of detection with conventional techniques. We plan to use single molecule super resolution imaging in conjunction with single molecule Förster Resonance Energy Transfer (smFRET) to overcome these limitations and provide insight into Homologous Recombination (HR) repair events at individual DSBs.

Sub-nanometer distance changes can be resolved via smFRET (**Fig. 1A**), which monitors the fluorescence of close proximity fluorophore pairs to determine dynamics and kinetics via the amount of Förster energy transfer, which in turn enables inference of conformational changes (**Fig. 1B**). During HR the DSB end "scans" the genomic DNA to find a complementary intact template for repair. Invasion of the template strand is initiated by RAD51, which forms a D-loop exposing the homologous acceptor strand. This search and strand invasion method of repair is arguably the most complicated DNA repair mechanism but is high fidelity and results in fewer mutations. However, neither the dynamics nor the sequence dependency of this process are well understood at the molecular level.

To investigate the sequence dependence of ssDNA/RAD51 strand invasion and stability, invading ssDNA oligos with different acceptor positions, end chemistries, nucleotide mismatch and reduced homology will be introduced to tethered template DNAs in the presence of RAD51 and ATP. The resulting FRET analysis will reveal the dynamic progression of homology sensing and strand invasion. Thus, determining the chemical and homology requirements for D-loop formation (**Fig 1 C &D**).

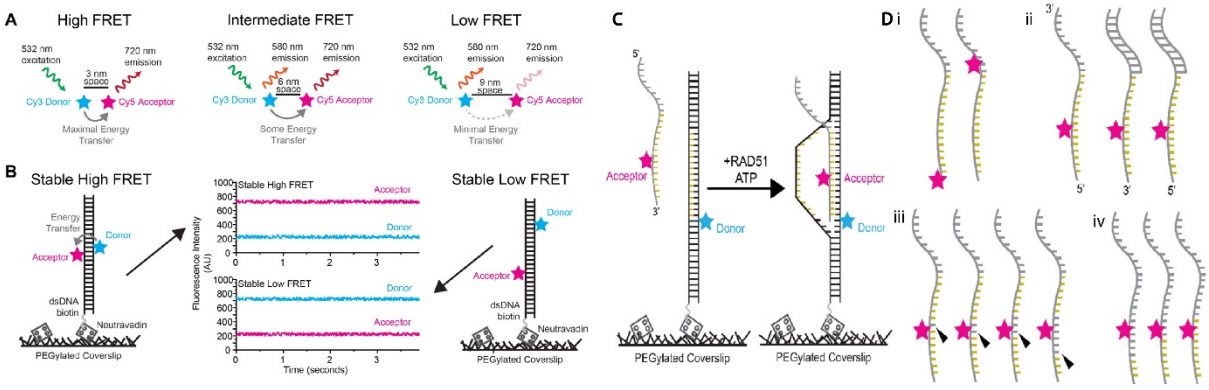

Figure 1 A. smFRET overview. **B.** Close proximty of the donor/acceptor (left) results in high acceptor signal (top-trace) whereas distal positioning (right) results in higher donor signal (bottom-trace). **C.** Addition of RAD51 allows the homologous acceptor-labeled DNA to form a D-loop, homologous sequence is shown in beige. **D.** Examples of proposed invading strands with **i.** different acceptor positions, **ii.** different end chemistries, **iii.** nucleotide mismatches at black arrows, and **iv.** reduced homolgy.