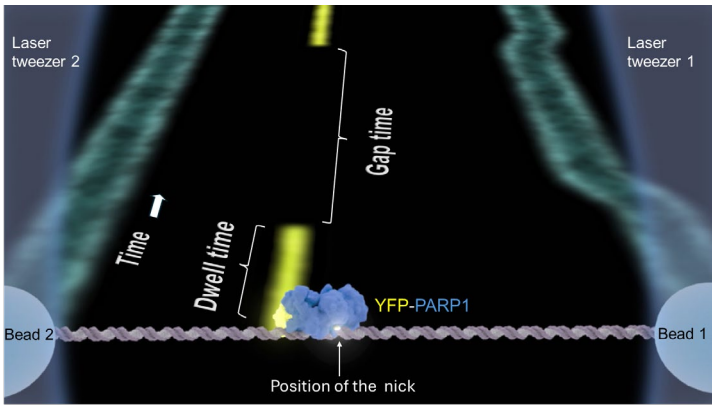
Single Molecule Analysis of DNA-binding Proteins from Nuclear Extracts

Bennett Van Houten, PhD, Richard M. Cyert Professor of Molecular Oncology, Department of Pharmacology and Chemical Biology, Co-Leader of the Genome Stability Program, UPMC-Hillman Cancer Center, University of Pittsburgh, PA

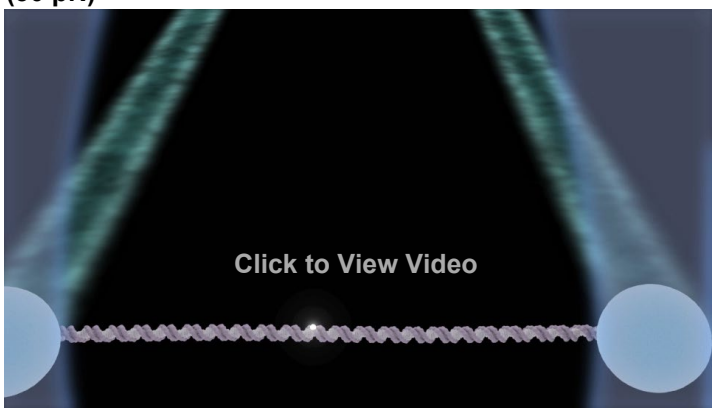
DNA repair is mediated by a highly choreographed dance of various proteins as they interrogate our genetic material for specific lesions and then process damaged bases to heal the DNA. By observing one molecule at a time, single-molecule studies can offer detailed insights about biomolecular processes including on rates, off rates, and diffusivity of molecules on strands of DNA However, in the past this approach requires purification and fluorescently tagging specific proteins of interest. This laborious process and the need for specialized equipment limits the general utility of single molecule fluorescence studies. A recent technological advance (Single-molecule Analysis of DNA-binding proteins from Nuclear Extracts, SMADNE) has lowered the barrier to entry for single-molecule studies, such that protein-DNA binding dynamics can now be determined directly out of nuclear extracts. In this approach, nuclear extracts containing fluorescently-tagged DNA repair proteins expressed in human cells following transient transfection are analyzed in a LUMICKS C-trap (*Nucleic Acids Research, 2023*). The C-trap uses correlative optical tweezers-fluorescence microscopy (CTFM) to follow up to three orthogonally labeled fluorescent proteins binding to DNA suspended between two beads. We were able to follow seven native DNA repair proteins and two structural variants, including: poly(ADP-ribose) polymerase (PARP1), heterodimeric ultraviolet-damaged DNA-binding protein (UV-DDB), and 8-oxoguanine glycosylase 1 (OGG1) on undamaged DNA on three forms of DNA damage. We discovered that PARP1 binds more quickly and longer to nicks when DNA is under physiological tension (**see Figure**) which we believe is due to the base stacking of Phe44 in Zinc finger 1. To compare and contrast the single-molecule DNA binding dynamics in nuclear extracts versus purified proteins experiments were performed with purified GFP-tagged 8-oxoguanine glycosylase 1 (OGG1), purified GFP-OGG1 spiked into nuclear extracts, and nuclear extracts from human cells overexpressing GFP-OGG1 (*DNA Repair,* 2024). We observed differences in OGG1 binding to undamaged DNA during DNA damage search in each of the three conditions. Purified GFP-OGG1 engaged undamaged DNA for a weighted average lifetime of 5.7 s and 21% of these events underwent DNA diffusion after binding. However, unlike other glycosylases studied by SMADNE, OGG1 does not bind non-damaged DNA efficiently in nuclear extracts. GFP-OGG1 binding dynamics on DNA substrates containing 8-oxoG were relatively similar in all three conditions, with the weighted average binding lifetimes varying from 2.2 s in nuclear extracts to 7.8 s with purified GFP-OGG1 in isolation.

Finally, we compared the purified protein and nuclear extract approaches for a catalytically dead OGG1 variant (GFP-OGG1-K249Q). This variant greatly increased the binding lifetime to 8-oxoG, with the weighted average lifetime for GFP-OGG1-249Q in nuclear extracts at 15.4 s vs 10.7 s for the purified protein. We believe SMADNE will provide a new window of observation into the behavior of nucleic acid binding proteins only accessible by biophysicists trained in protein purification and protein labeling. This work is supported by a generous grant award (R35ES031638) from NIEHS,NIH and an equipment grant (S10OD032158) from NIH.

Kymograph of YFP-tagged PARP1 binding and rebinding to a nick (yellow time streak)



YFP-PARP1 binds nicked DNA better under high tension (30 pN)



Cartoon of a single molecule of YFP-PARP1 binding to a nick. Using a LUMICKS C-trap, the biotinylated-DNA containing nicks is captured to two streptavidin beads suspended in two optical traps (laser tweezer). Bead 1 can be manipulated with a joy stick and tension on the DNA can be increased by pulling on bead 1 and extending the length of the DNA. Under tension PARP1 binds more rapidly and longer to nicks. The time in between binding events, gap time, is a measure of the on rate, and the dwell time is a measure of the off rate of PARP1 to DNA. When ever YFP-PARP binds to a nick it generates a yellow time streak which is called a kymograph. A laser scans the DNA at 10 times/second generating the kymograph.