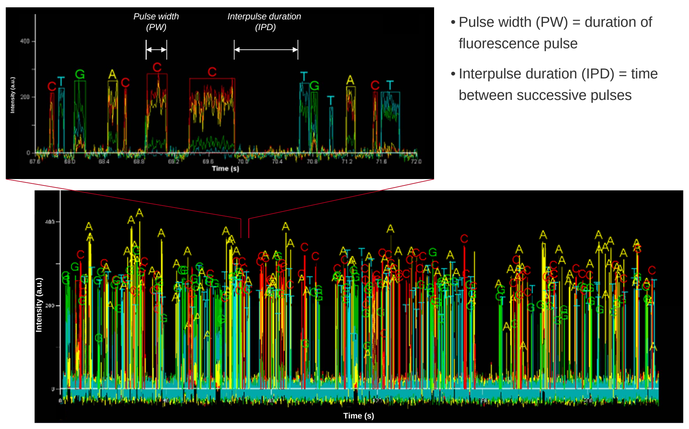
Direct Sequencing and Identification of Damaged DNA Bases

Tyson A. Clark[1], Khai Luong[1], Kristi Spittle[1], Jessica Lee[1], James Bullard[1], Patricia Respuela[2],
Eric Schadt[1], Stephen W. Turner[1], Ashby Morrison[2], and Jonas Korlach[1]
[1]Pacific Biosciences, 1380 Willow Road, Menlo Park, CA 94025 [2]Stanford University, 371 Serra Mall, Stanford, CA 94305

Abstract

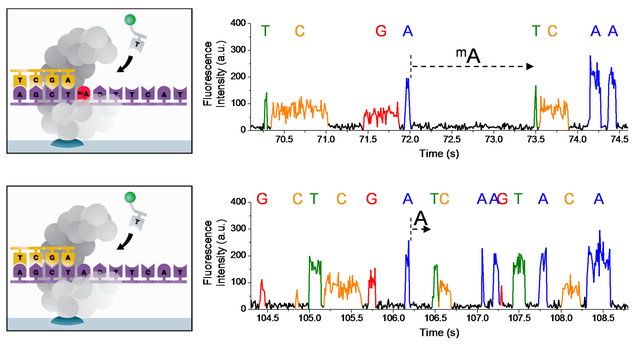
DNA is under constant stress from both endogenous and exogenous sources. DNA base modifications resulting from various types of DNA damage are wide-spread and play important roles in affecting physiological states and disease phenotypes. Examples include oxidative damage (8-oxoguanine, 8-oxoadenine; aging, Alzheimer's, Parkinson's), alkylation (1-methyladenine, 6-O-methylguanine; cancer), adduct formation (benzo[a]pyrene diol epoxide (BPDE), pyrimidine dimers; smoking, industrial chemical exposure, chemical UV light exposure, cancer), and ionizing radiation damage (5-hydroxycytosine, 5-hydroxyuracil, 5-hydroxymethyluracil; cancer). Currently, these and other products of DNA damage cannot be sequenced with existing sequencing methods. In contrast, single molecule, real-time (SMRT®) DNA sequencing can report on modified DNA bases through an analysis of the DNA polymerase kinetics that is affected by a modified base in the template. We demonstrate the DNA strand-resolved sequencing of over 8 different DNA-damage associated base modifications, with base pair resolution and single DNA molecule sensitivity. We also report on the application of this sequencing capability to biological samples and the development of a generic, open-source algorithm to analyze kinetic information from SMRT sequencing.

Detection of Base Modifications by SMRT® Sequencing

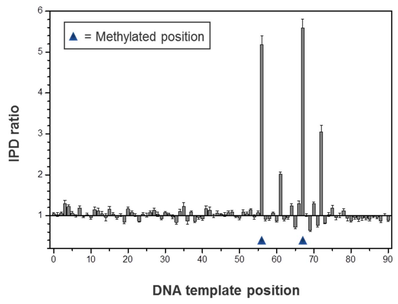


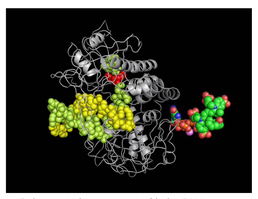
- Pulse width (PW) = duration of fluorescence pulse
- Interpulse duration (IPD) = time between successive pulses

Effects of Base Modifications on Polymerase Kinetics:



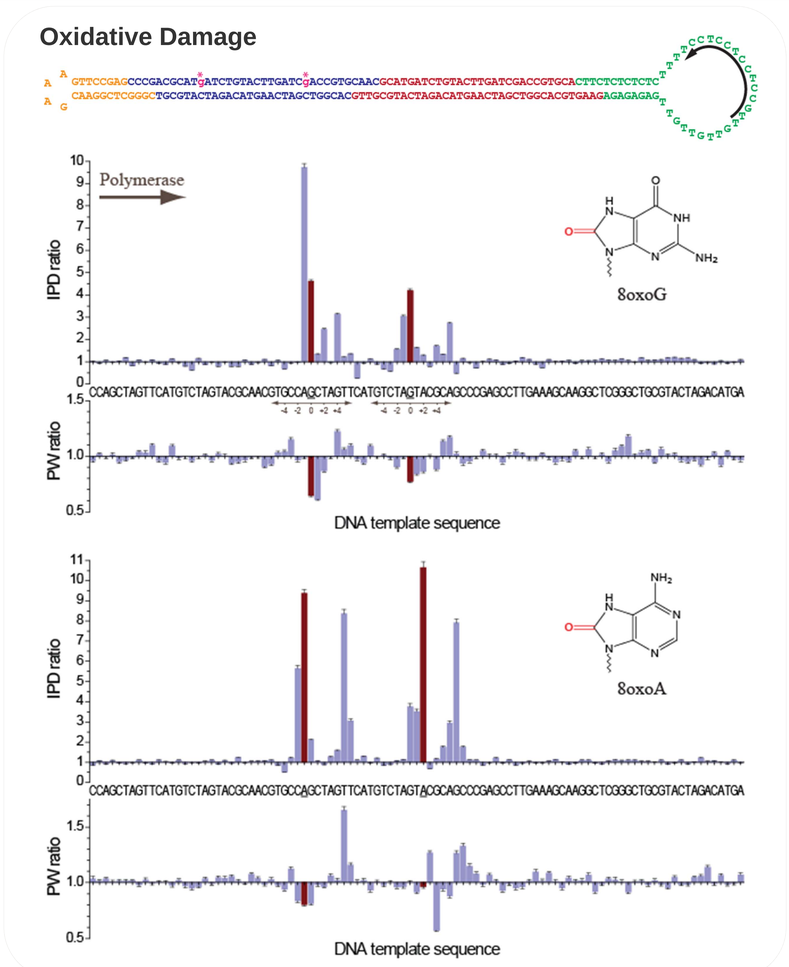
IPD is increased before T incorporation across N6-methyladenine (^{m}A)

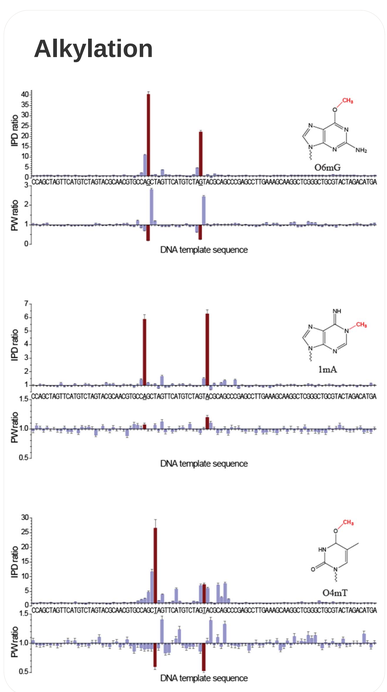


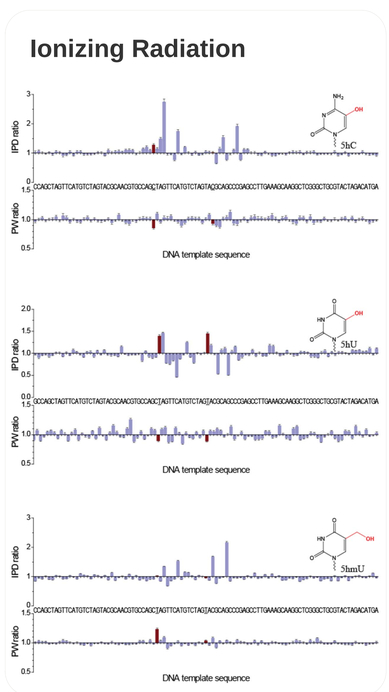


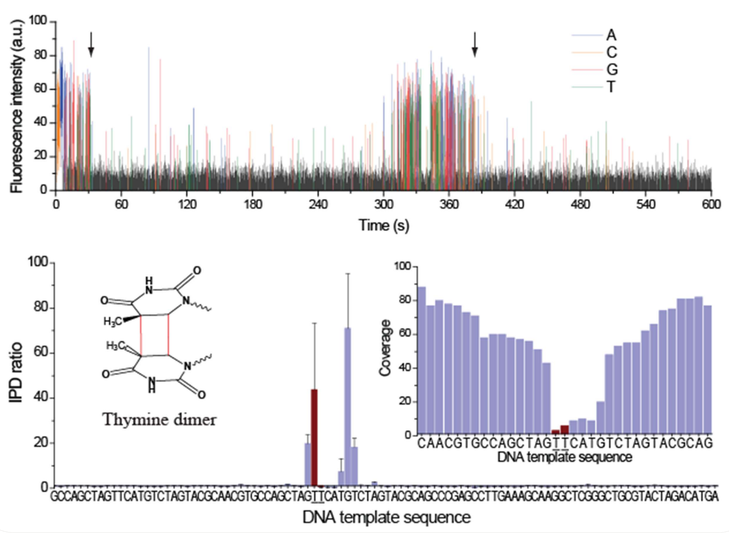
Polymerase has contacts with the DNA modification before and after incorporation of the base opposite the modification

Kinetic Effects of Damaged DNA Bases

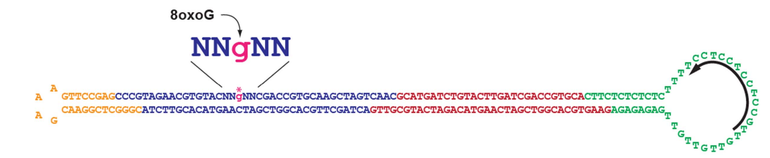


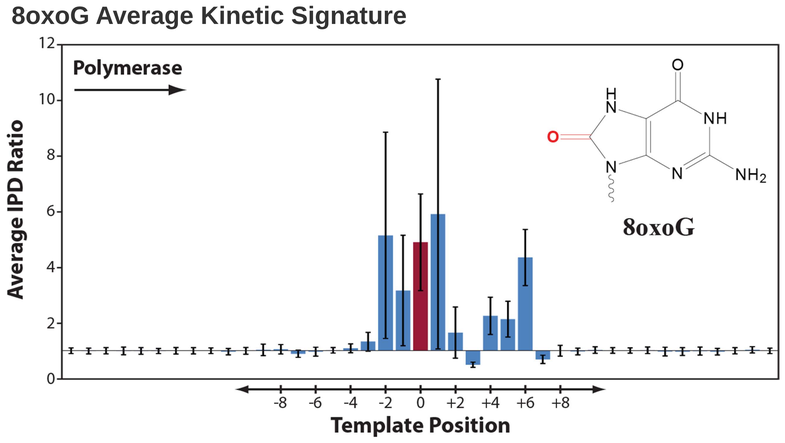


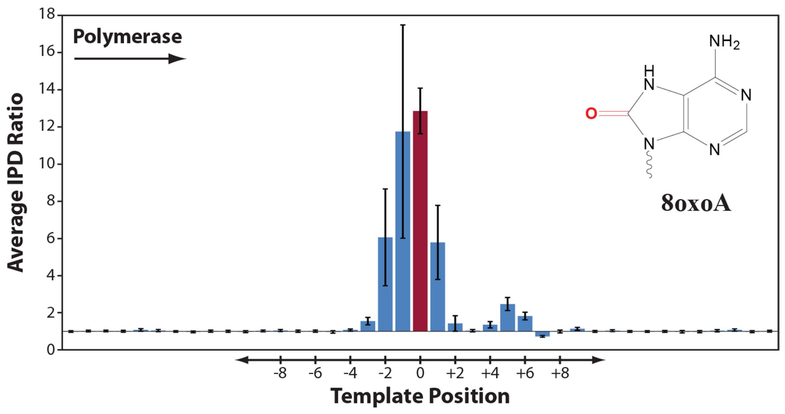


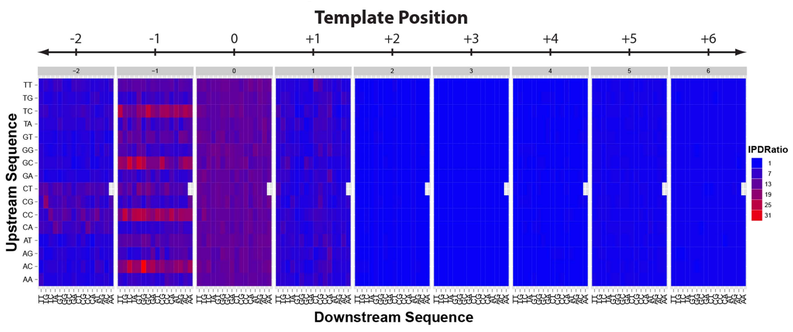


Sequence Context Dependence









Conclusions and Future Directions

- Various types of DNA damage result in base modifications that can be detected during SMRT sequencing
- Each modification has a distinct kinetic signature
- Kinetic signatures vary based on surrounding sequence context
- Algorithms are being developed to distinguish the modification types and better understand the nature of the sequence-context dependence on IPD
- Detection of DNA damage is being extended to the genome scale looking at 8-oxoG in yeast cells treated with methylene blue and light to induce oxidative damage

References

Flusberg, *et al.* "Direct detection of DNA methylation during single-molecule, real-time sequencing." *Nature Methods*. 2010 Jun;7(6):461-5.

Clark, *et al*. "Direct detection and sequencing of damaged DNA bases." *Genome Integrity*. 2011 Dec 20;2:10.