

Materials Technology Center Seminar Series

Monitoring the Base-Excision Repair Activity of Uracil-DNA Glycosylase Using an α -Hemolysin Nanopore

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Nanopores have been investigated as a simple and label-free tool to characterize DNA nucleotides when a ssDNA strand translocates through the constriction of the pore. Here, a wild-type α -hemolysin protein nanopore was used to monitor DNA repair enzyme activity based on base-specific interactions of dsDNA with the vestibule constriction “latch”, a previously unrecognized sensing zone in α -hemolysin specific for dsDNA structure. The presence of a single abasic site within dsDNA that is in proximity of the latch zone results in a large increase in ion channel current, allowing accurate quantitation of the kinetics of base repair reactions involving an abasic site product. Taking advantage of the high resolution for abasic site recognition, the rate of uracil-DNA glycosylase hydrolysis of the *N*-glycosidic bond, converting 2'-deoxyuridine in DNA to an abasic site, was continuously monitored by electrophoretically capturing reaction substrate or product dsDNA in the ion channel vestibule. Our results can be adapted to monitor the activity of other enzymes that introduce a change in the oligonucleotide structure, and thus provide a new approach for monitoring enzymatic activity on DNA. The discovery of a very sensitive sensing zone at the latch suggests the potential development of new methods to detect site-specific changes in dsDNA structure relevant to epigenetic, forensic and medical diagnostic applications.

Wednesday, October 23
4:00-5:00 p.m.
Neckers 240