DEVELOPMENT OF OPTICAL TRAPPING TECHNIQUES IN PREPARATION FOR MECHANISTIC UDG STUDIES

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Abstract

Uracil DNA Glycosylase (UDG) repairs damaged DNA by enzymatically excising uracil from DNA and replacing it with an appropriate nucleotide. While two proposed mechanisms for the location of damaged nucleotides exist, the full kinetic and mechanistic details remain unknown. Previous single-molecule fluorescence studies have shown that fluorescently tagged UDG can be observed binding to damaged DNA in real time. However, these studies utilized damaged DNA resting in physical contact with a microscope slide. The proximity of the DNA to the slide surface led to a lack of reproducibility in experimental results. Thus, we have begun work to suspend a strand of DNA above a surface, which will give UDG three-dimensional, unrestricted access to the damaged DNA. The approach described here involves creating a complex that resembles a dumbbell, with a strand of lambda DNA suspended between two polystyrene beads above a surface. To date, we have installed the necessary microinjection, optical trapping, bead manipulation instrumentation. Preparations are currently underway to synthesize a bead-DNA-bead construct and chemically attach it to a surface.

Category

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