Research Highlight #300

Soma Dhakal, Ph.D.
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Gaining a single-molecule understanding of the RuvA-Holliday-junction complex: a key player in repairing DNA during homologous recombination.

Dr. Dhakal's research group is one of the first to conduct a mechanochemical study of DNA Holliday junctions (HJs) and the RuvA-HJ complex: a crucial protein-DNA complex formed during the DNA damage repair process called homologous recombination. Homologous recombination is a well-conserved pathway that cells use to repair double-stranded DNA breaks and has important implications for genetic health. Dr. Dhakal's research focuses on gaining a mechanistic understanding of how these DNA damage repair proteins bind to the HJ by observing the resulting conformations and measuring their stability.

> "We are using optical tweezers for unfolding a DNA secondary structure, specifically, a four-way DNA junction called the Holliday junction, which is important because it is formed during the DNA damage repair process called homologous recombination. This has biological applications as there are a specific set of proteins that recognize the junction, bind to it, and resolve it during the DNA repair process. We are interested in having some mechanistic understanding of how these DNA damage repair proteins bind to that junction and what they do in terms of the four-way DNA conformations and stability. The other application that we have been pursuing is looking at the mechanical properties of protein filaments in a completely different project."

Their interest in understanding the mechanisms behind the Holliday junction, which is such an important intermediate and player in the biological maintenance of DNA, is what led Dr. Dhakal and his lab to use optical tweezers, an instrument that provides force sensing, optical trapping, and manipulation capabilities. They use optical tweezers to study protein-DNA interactions and to quantify the forces associated with unfolding events.

Using fluorescent microscopy and optical tweezers

Dr. Dhakal and his lab found that using optical tweezers was a useful approach for unfolding the RuvA–Holliday-junction complex and measuring the resulting unfolding force. This force is a measure of the mechanical stability of the protein-DNA complex at a given pulling speed and is a good indicator of how proteins will interact with a specific DNA structure under stress. Dr. Dhakal has extensive experience using both optical tweezers and fluorescent microscopy after studying DNA secondary structures with custom-built optical tweezers during his Ph.D. Afterwards, during his post doc, he utilized single-molecule fluorescence microscopy to visualize protein DNA

ABOUT THE RESEARCHER

Soma Dhakal, Ph.D., is an assistant professor at the Virginia Commonwealth University in Richmond, Virginia where he leads the "Single Molecule Microscopy and DNA Nanotechnology Lab." Dr. Dhakal earned a B.S. and M.S., from Tribhuvan University, a Ph.D., at Kent State University, and completed his Postdoctoral Research Fellow at the University of Michigan, Ann Arbor. In his Ph.D. and postdoctoral research, he studied the unfolding of various DNA secondary structures using optical tweezers and protein-DNA interactions as well as functional studies of single enzymes using single-molecule fluorescence microscopy.

Website: Single Molecule Microscopy and DNA Nanotechnology Lab

Recent Publications:

Dalton, GR., Roaa, M., Anisa, K., Soma, D. (2021). Direct unfolding of RuvA-HJ complex at the single-molecule level. *Biophysical Journal,* 120(10): 1894-1902, https://doi.org/10.1016/j.bpj.2021.03.006

> "The optical tweezers really helped us visualize the reformation of the junction with RuvA, which is a very new insight for the DNA damage repair field."

interactions by making DNA origami, among other lab techniques. Most recently, Dr. Dhakal made excellent use of Bruker's NanoTracker2, a dual-beam optical tweezers setup with compact hardware.

> "These optical tweezers have a very high force-distance sensitivity, basically down to sub-picoNewton force, and nanometer-level distance sensitivity. It's really cool because we are looking at the protein-DNA interaction that is occurring at the nanometer scale. These tweezers are very easy to use and most of the programs are written very well and are user-friendly. We also found that these tweezers are very reliable and have a pretty stable system that is packaged well in a small configuration that doesn't take up much space in the lab."

Despite the difficulty of targeting a single molecule with optical tweezers — a skill that Dr. Dhakal says takes a lot of patience to master — the Virginia Commonwealth group was able to uncover invaluable insights into RuvA–Holliday-junction interactions.

Single-molecule data

Dr. Dhakal and his research group found that it takes a much higher force to unfold the HJ when RuvA is present, which is what they expected. However, they were surprised to find that when they relaxed the force, the proteins remained bound to the mechanically melted HJ and the DNA junction was reformed at an unusually high force, leading to the conclusion that the RuvA protein is actually stabilizing the HJ. Additionally, they were able to show a high level of reproducibility from molecule to molecule, which gives them confidence in the interpretation of their results.

Looking toward future studies in humans

This research group used optical tweezers to study the 4-way DNA Holliday junction and RuvA, but Dr. Dhakal mentions that their platform can be used for studying various Holliday junction binding proteins. Specifically, they have started looking into the behavior of human proteins such as RAD51B and RAD54 by manipulating and moving the proteins in different directions to understand and measure their critical roles in stabilizing DNA and various structures formed during the repair pathway. Dr. Dhakal elaborates on this by explaining what they will be working on in the future.

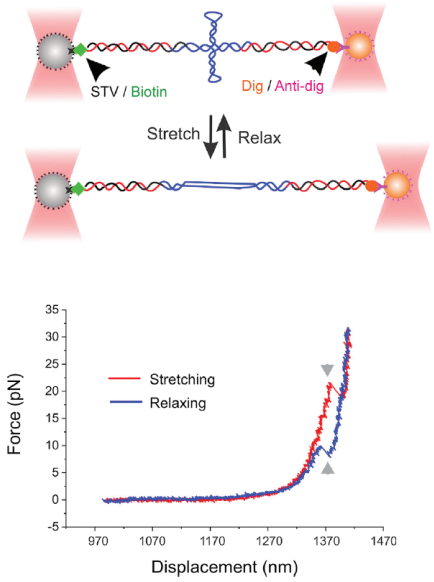

Figure 1.

Schematic of the optical tweezers setup (top) and a typical force versus extension (*F-X*) curve for the HJ construct with unfolding/refolding events (bottom).

> "The hope is that it [future protein-HJ studies] will provide an unprecedented mechanistic understanding of how proteins operate on the junction, which is actually considered the central intermediate of this entire repair process. Hopefully, in the future, this knowledge will contribute to developing some therapeutic drugs."

Having a mechanochemical understanding of how the prokaryotic protein RuvA binds to and impacts the unfolding/refolding of the Holliday junction provides important insights into the homologous recombination process. By utilizing both single-molecule fluorescence microscopy and optical tweezers, Dr. Dhakal's research group has collected and presented data in the *Biophysical Journal* that makes them one of the first to manipulate the crucial protein-Holliday-junction complex at the single-molecule level. Additionally, the single-molecule platform they created can also be easily adapted to investigate many other HJ-binding proteins in future studies.

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