A more realistic look at DNA in action: Study shows it behaves differently when crowded by molecules

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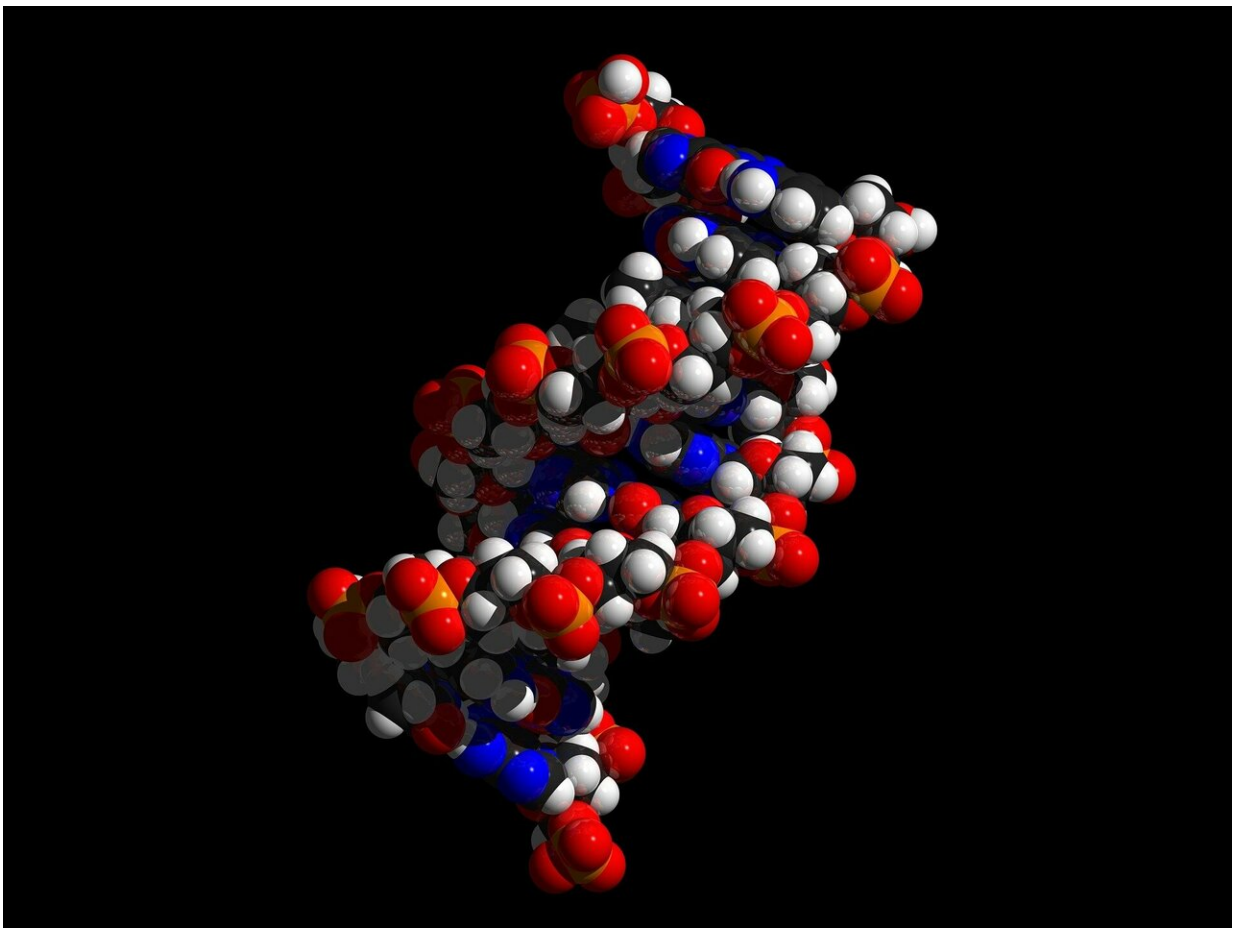

Credit: CC0 Public Domain

By creating a more true-to-life representation of DNA's environment, researchers at Northwestern University have discovered that strand separation—the essential process a "resting" double helix undergoes before it can initiate replication or make repairs—may take more mechanical force than the field previously believed.

Most biochemistry labs that study DNA isolate it within a water-based solution that allows scientists to manipulate DNA without interacting with other molecules. They also tend to use heat to separate strands, heating the DNA to more than 150°F, a temperature a cell would never naturally reach. By contrast, in a living cell DNA lives in a very crowded environment, and special proteins attach to DNA to mechanically unwind the double helix and then pry it apart.

"The interior of the cell is super crowded with molecules, and most biochemistry experiments are super uncrowded," said Northwestern professor John Marko. "You can think of extra molecules as billiard balls. They're pounding against the DNA double helix and keeping it from opening."

Marko, a professor of molecular biosciences as well as physics in Northwestern's Weinberg College of Arts and Sciences, led the research along with Northwestern post-doctoral researcher Parth Desai.

In Marko's lab, for their experiments, he and Desai use microscopic magnetic tweezers to separate DNA and then carefully attach strands of it to surfaces on one end, and tiny magnetic particles on the other, then conduct high-tech imaging. The technology has been around for 25 years, and Marko was one of the first researchers theorizing about and then using it.

Marko and Desai wrote the paper that not only identifies but quantifies the amount of stress imposed by crowding, that will be published in the

Biophysical Journal.

Desai introduced three types of molecules to the solution holding DNA to mimic proteins and investigated interactions among glycerol, ethylene glycol and polyethylene glycol (each approximately the size of one DNA double helix, 2 or 3 nanometers).

"We wanted to have a wide variety of molecules where some cause dehydration, destabilizing DNA mechanically, and then others that stabilize DNA," Desai said. "It's not exactly analogous to things found in cells, but you could imagine that other competing proteins in cells will have a similar effect. If they're competing for water, for instance, they would dehydrate DNA, and if they're not competing for water, they would crowd the DNA and have this entropic effect."

While fundamental, research like this has "been the basis for many, many, many medical advances," Marko said, such as deep sequencing of DNA, where scientists can now sequence an entire human genome in under a day. He also thinks their findings may be broadly applicable to other elements of fundamental biochemical processes.

"If this affects DNA strand separation, all protein interactions with DNA are also going to be affected," Marko said. "For example, the tendency for proteins to stick to specific sites on DNA and to control specific processes—this is also going to be altered by crowding."

In addition to running more experiments that incorporate multiple crowding agents, the team hopes to move closer to a true representation of a cell, and from there, study how interactions between enzymes and DNA are impacted by crowding.

More information: Parth Rakesh Desai et al, Molecular Crowding Suppresses Mechanical Stress-Driven DNA Strand Separation,

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