A vital game of hide-and-seek elucidated by novel single-molecule microscopy

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Life depends on an intricate game of hide-and-seek taking place inside the cell. New research, now published in the journal *Nature*, sheds light on the mechanisms with which DNA-binding proteins search the genome for their specific binding sites.

DNA is a double-helical molecule that stores all the instructions a cell requires to sustain itself. The information is encoded within the specific

sequential order of genetic letters (the DNA base pair sequence). Correctly implementing the vital instructions stored in this genetic code depends on the ability of proteins to recognize and selectively bind to specific DNA sequences. Such proteins include transcription factors, which have the crucial task of switching genes on and off by binding to specific transcription factor binding sites. Failure to engage these DNA target sites at the right place and time would have disastrous consequences for cellular life—genes would not be switched on when needed, while others might never turn off.

From the perspective of a transcription factor, finding its specific binding site amounts to finding the proverbial needle (i.e., short stretch of DNA, often around a dozen genetic letters only) in a haystack (the genome, ranging from millions to billions of letters depending on the organism). This so-called search problem has been studied extensively, and many proteins utilize a process termed facilitated diffusion to accelerate their search. Here, a protein undergoes three-dimensional diffusion (Brownian motion) until it randomly bumps into a DNA molecule. If the site of collision does not correspond to the correct binding site, the protein can undergo 1-D diffusion by randomly sliding back-and-forth along the DNA before unbinding and returning to 3-D diffusion. Scientists have long established that the 1-D sliding process accelerates the search, but the precise mechanism of 1-D sliding has remained enigmatic.

In this new study, led jointly by Uppsala University researchers Sebastian Deindl and Johan Elf, the 1-D sliding mechanism takes center stage.

"The molecular mechanism of the scanning process has been poorly understood, and it has remained a great mystery how transcription factors manage to slide fast on non-specific DNA sequences, yet at the same time bind efficiently to specific targets," says Ph.D. student and

joint first author Emil Marklund.

In order to tackle these questions, the two research teams developed new fluorescence microscopy imaging approaches to observe individual transcription factor proteins sliding along the DNA in real time as they search for and bind to the correct binding site.

"It is exciting that we were able to develop new imaging approaches to directly observe, for the first time, if and how often the sliding protein fails to recognize and slides past its binding site," says Deindl.

It turns out the sliding protein is quite sloppy and frequently misses its target site. In order to better understand how the sliding protein explores the DNA surface, a new way of tracking and shooting extremely fast movies of the rapidly sliding protein had to be developed. The protein searches the DNA very fast: 10 base pairs are scanned in around 100 microseconds (one microsecond corresponds to one millionth of a second). The researchers realized they needed to carry out much faster measurements than anyone had done before to investigate how the protein explores the DNA surface on these length- and timescales.

Using this new microscopy approach, the authors could follow the sliding protein's helical path around the DNA molecule.

"It's great that we can push the dynamic observation of bimolecular interactions to the sub-millisecond time scale—this is where the chemistry of life happens," says Elf.

The sliding protein turned out not to strictly follow the track given by the helical geometry of the DNA molecule itself. Instead, it was observed to slip out of its track quite frequently by making short hops.

"By hopping, the protein trades thorough scanning for speed, so it can

scan DNA faster. This is a really smart choice by the protein, since it will find the target twice as fast using this search mechanism," says Marklund.

More information: DNA surface exploration and operator bypassing during target search, *Nature* (2020). DOI: 10.1038/s41586-020-2413-7 , www.nature.com/articles/s41586-020-2413-7

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