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A Dynamic Single-Molecule Approach to Directly Visualize the Molecular Mechanisms of DNA-Binding Proteins.

Studying and validating the molecular mechanism of DNA processes often requires direct functional evidence. Here we present the C-Trap: a dynamic single-molecule microscope that allows researchers to directly visualize the dynamics and assembly of biological complexes under different conditions. All at the single-molecule level, providing direct proof of the biological mechanisms being studied.

Fully understanding the mechanism underlying DNA repair, transcription, editing, or organization requires a multidisciplinary approach. Structural techniques such as cryo-EM reveal unprecedented detail into the structure of the proteins, but they are static representations and do not provide direct functional evidence. On the other hand, bulk biochemical assays provide insights into the function, but the outcomes are averaged in time and over many molecules, hindering information regarding the exact effect of different actors in the molecular mechanism. By adding dynamic single-molecule information one gains direct proof of how each component works together in the molecular machinery.

Here, we present our efforts to further enable discoveries in the field of DNA-protein interactions and DNA organization using the combination of optical tweezers with correlative confocal fluorescence microscopy. We present several examples in which our technologies enhanced the understanding of the DNA repair mechanisms, chromatin structure and DNA editing tools. Furthermore, we show that advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that has the ability to open up new venues in the field of DNA-protein interactions.

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