Filming single proteins at work using high-speed atomic force microscopy

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Structural biology has long been contributing to our understanding of how proteins function by solving their detailed structures. However, the revealed structures were restricted to static information. This restriction is now removed by high-speed atomic force microscopy (HS-AFM) that enables us to directly visualize individual proteins in action. HS-AFM studies provided new mechanistic insight into the functional mechanism of proteins [1-3]. For example, HS-AFM movies of bacteriorhodopsin (bR), which functions as a light-driven proton pump, clearly showed that, upon illumination, a cytoplasmic portion of bR displaced toward adjacent molecules at ~ 0.7 nm [1]. Therefore, high-resolution visualizations of protein dynamics are powerful approaches for studying elaborate biomolecular processes under realistic conditions.

In this study, we applied HS-AFM to visualize the real-space and real-time dynamics of CRISPR-Cas9, which has been widely used for numerous applications, such as genome editing. The CRISPR-associated endonuclease Cas9 binds to a guide RNA and cleaves double-stranded DNA with a sequence complementary to the RNA guide. Our HS-AFM movies showed that apo-Cas9 adopts unexpected flexible conformations, while Cas9–RNA forms a stable bilobed structure and interrogates a target site on the DNA by three-dimensional diffusion. These HS-AFM movies also provided real-time visualization of the Cas9-mediated DNA cleavage reaction. Notably, the Cas9 HNH nuclease domain fluctuates upon DNA binding, and subsequently adopts an active conformation, where the HNH active site is docked at the cleavage site in the target DNA. Collectively, our HS-AFM data extend our understanding of the action mechanism of CRISPR-Cas9 [3].

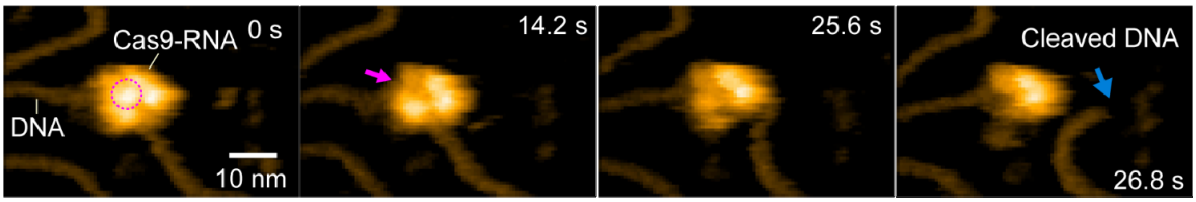

Figure. A sequential HS-AFM images of a Cas9–RNA–DNA during DNA cleavage.

References

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