Single-molecule visualization for probing molecular mechanisms of proteins that maintain genomic stability

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Single-molecule visualization is a powerful tool to probe the molecular features that cannot be discoved by traditional methods. Here we introduce how a single-molecule technique called DNA curtain reveals the molecular details of proteins that maintain genomic stability.

Firstly, we study human XPC-RAD23B that finds DNA lesions and recruits downstream factors in nucleotide excision repair (NER). We observe the heterogeneity in motions of XPC-RAD23B, exhibiting diffusive, constrained, and immobile states. We find that the heterogeneity results from the interaction between hXPC-Rad23B and DNA breathing in AT- rich regions. We also find that the diffusion coefficient dramatically increases according toionic strength, suggesting that hXPC-RAD23B diffuses along DNA via hopping, facilitating to bypass protein obstacles on DNA. Secondly, we investigate the structural and biochemical features of a bromodomain-containing AAA+ ATPase, Abo1. We reveal the cryo-EM structure of Abo1, displaying a closed hexameric ring structure. We observe the conformational change of Abo1 depending on ATP hydrolysis in real time using a high-speed AFM, which demonstrates that subunits of Abo1 stochastically hydrolyze ATP. Furthermore, using the DNA curtain assay, we manifeste that Abo1 does not dislodge but assembles histones depending on ATP hydrolysis.

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