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Monitoring of protein function at single-molecule level with single-event precision using fluorescence microscopy imaging DNA CueR Cy3Cy5

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Recent advances in microscopy-imaging and discovery of new type of fluorescent dyes have made it possible to monitor the function of proteins at single molecule level with single event precision. In this study, we probed the dynamics of the fluorescent-dye labeled protein-DNA interactions using two-color total international reflection fluorescence microscopy (TRIM) technique to explore the metal regulation phenomena. Some of the metals are essential, but many are toxic to organisms. Even the essential metals, for example iron and copper, can become harmful above a certain concentration inside cells. Many biological processes regulate and maintain intracellular metal quota. One of them is through metalloregulators, which respond to metal ions and regulate the transcription of genes that protect the bacteria from metal-induced stress. We studied the dynamic interactions of the copper efflux regulator (CueR), a Cu-responsive MerR-family metalloregulator, with DNA using single-molecule biophysical technique. Besides quantifying its DNA binding and unbinding kinetics, we discovered that CueR also has two different binding modes, corresponding to interactions with specific and nonspecific DNA sequences, which would facilitate recognition localization. Most strikingly, a CueR molecule coming from solution can directly substitute for a DNA-bound CueR or assist the dissociation of the incumbent CueR, both of which are unique examples for any DNA-binding protein. The kinetics of the direct protein substitution and assisted dissociation reactions indicate that these two unique processes can provide efficient pathways to replace a DNA-bound holo-CueR with apo-CueR, thus turning off transcription promptly and facilely. This methodology opens ways to explore cellular regulation mechanism at unprecedented single-molecule and single-event precision.

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