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Real-time feedback-driven single-particle tracking spectroscopy of LHCII

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Single-molecule spectroscopy (SMS) has proven to be a powerful technique for investigating structure-function relationships in light-harvesting systems. In particular, SMS has unraveled dynamics in light-harvesting complexes that are hidden in ensemble measurements. However, the environment used in SMS experiments is a poor representation of the natural cellular environment, and therefore the results of these studies may be of limited physiological relevance. One limitation of conventional SMS experiments is the need to immobilize the complexes via surface attachment or to trap the complexes using, e.g., an anti-Brownian electrokinetic (ABEL) trap. This limitation is overcome by real-time feedback-driven single-particle tracking (RT-FD-SPT), a non-invasive technique that allows SMS measurements to be performed on single, freely diffusing particles for extended durations and with excellent spatiotemporal resolution. We studied different RT-FD-SPT methods using statistical analysis and simulations before using RT-FD-SPT to experimentally measure fluorescence lifetimes and emission spectra of single diffusing plant LHCII complexes. This paves the way for studies of the effect of surface immobilization as well as for studying single LHCII complexes in close-to-natural environments.

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