Fluorescent Cinchona Alkaloids for Single Molecule Studies of Organocatalysis

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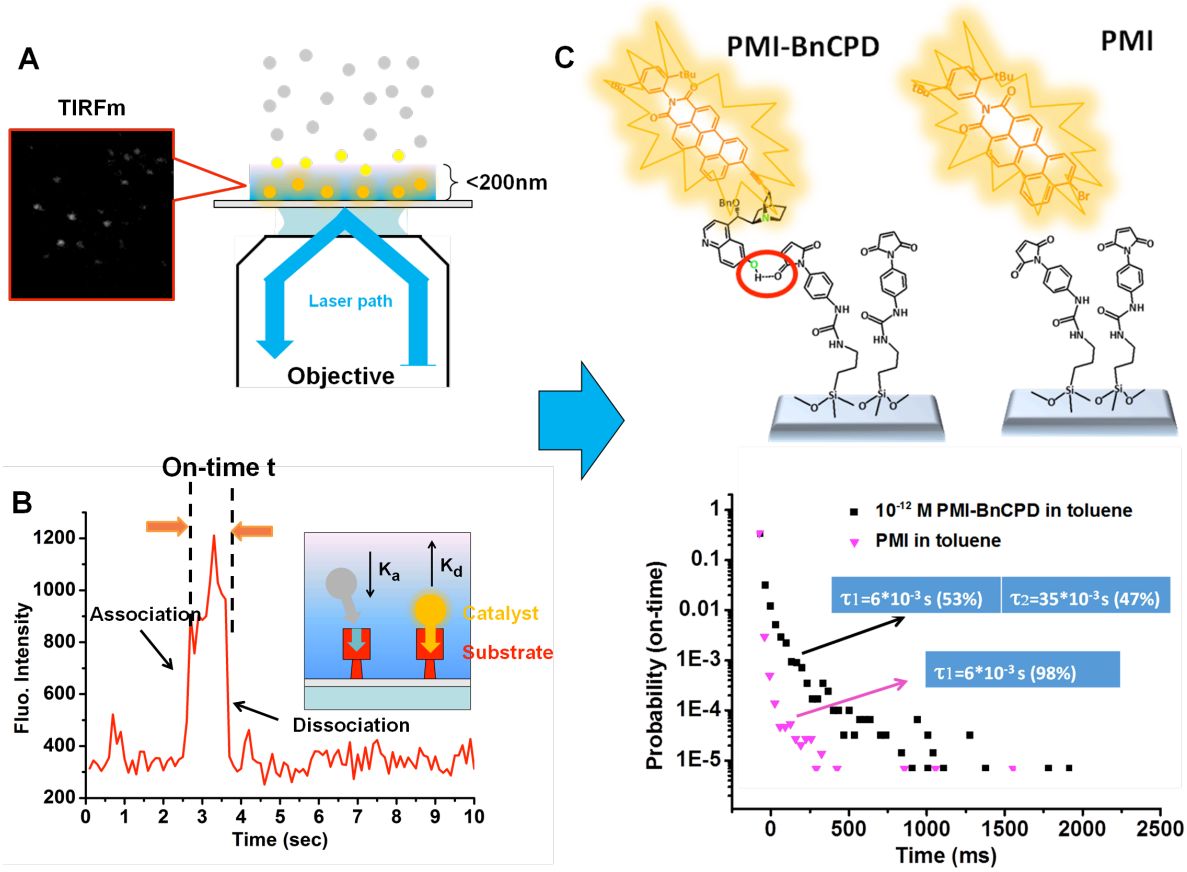

Figure 1. Examples of single-molecule fluorescence microscopy studies of catalytic chemical reactions. A) Principle of TIRF microscopy, B) Fluorescence time trace of a representative individual molecule. C) Cinchona catalyst with PMI (or PMI reference compound without the catalytic group) interacting with the maleimide functionalized coverslip (top), comparison of the probability histogram of the on-time Δt from single-molecule hydrogen bonding experiments (bottom).

Single-molecule fluorescence due to its potential to detect rare events has been proven useful for the investigation of catalytic reactions, e.g. with enzymes and zeolites.[1, 2, 3, 4] To gain new insights into asymmetric organocatalysis reactions, and further understand their mechanisms, we studied the reaction between a thiol and maleimide, catalyzed by cinchona alkaloids and other organocatalysts. Single molecule techniques are applied in this project to obtain dynamic information which is unavailable in ensemble experiments. The general approach used in our experiments is shown schematically in Fig. 1. Firstly, we examined the binding

affinity of the PMI-tagged cinchona alkaloid organocatalyst (PMI-BnCPD) to a maleimide (PMPI), which immobilized on glass microscope coverslip (Fig. 1C bottom). It is anticipated that PMI-BnCPD can rapidly and reversibly bind with surface maleimide through hydrogen bonding from the catalyst's hydroxyl group, or by nucleophilic addition of the amino group. Intensity changes in individual fluorescent spots were recorded (Fig. 1B) under TIRF illumination and sCMOS camera detection (Fig. 1A). The single-molecule signal was extracted and tracked using a home made Matlab program based on the *'The matlab particle tracking code repository'*.[2] For kinetic analysis, the on-time Δt of each single molecule was rescaled by the time constant of 34 ms/ frame and collected in a histogram normalized to the probability density distribution shown in Fig. 1C (bottom). A model function with bi-exponential decay was fitted to the data to estimate the time constants of 3.5×10^{-2} s (47%) and 6×10^{-3} s (53%), which we attribute to bonding between cinchona and maleimide and non-specific binding to the surface, respectively.

As a next step we will introduce thiols as the second reactant to further study this asymmetric cinchona catalysis. In the near future, we hope to directly observe catalytic events at the single molecule level and to obtain intermediate state information of the organic reaction process.

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