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Development of dynamic quenching based two-dimensional fluorescence lifetime correlation spectroscopy for studying microsecond conformational dynamics of singly labeled biopolymers

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[Abstract] Single molecule FRET (smFRET) is widely employed to study conformational dynamics of biopolymers. FRET requires multiple dye labeling and provides conformational information through the distance between two dyes. Here, we report a new method to study microsecond-resolved conformational dynamics of biopolymers with only single dye labeling by combining dynamic fluorescence quenching and two-dimensional fluorescence lifetime correlation spectroscopy (DQ 2D FLCS). Dynamic quenching makes the fluorescence lifetimes of individual conformers different when the solvent accessibility around the dye is different. 2D FLCS distinguishes these conformers and provides their interconversion timescale with μs-time resolution. Applying DQ 2D FLCS to a singly-labeled hairpin DNA, we successfully resolved ~50 μs dynamics between the 'open' and 'folded' states. This new method can be utilized to study dynamic changes in the local environment of a biopolymer, which is complementary to the information about global dynamics obtained with smFRET.

[Introduction] The functions of biopolymers, such as proteins or polynucleotides, are governed by their conformations, and more importantly, by fluctuations among the conformational ensembles. In contrast to ensemble measurements which observe the average behavior of biopolymers, single molecule measurements can detect individual conformers and their interconversion dynamics. However, conventional smFRET methods can usually probe conformational transitions only on a ms and slower timescales. Moreover, FRET requires multiple fluorophore labeling and is sensitive only for a limited range of dye-pair distance.

In this work, we report on a single-dye based single molecule method by employing 2D FLCS and dynamic fluorescence quenching. 2D FLCS is a novel single molecule tool that distinguishes the conformers based on their fluorescence lifetimes, and it provides μs-resolved reaction kinetics [1]. Dynamic quenching is a process in which some external quenchers (e.g. iodide ion) non-radiatively de-exite a fluorescent dye by collision [2]. Because dynamic quenching results in shortening of the fluorescence lifetime of a dye, if it is applied to a dye-labeled biopolymer, different conformers become distinguishable with different fluorescence lifetimes reflecting different solvent accessibilities around the dye. We named this new method "dynamic-quenching based 2D FLCS (DQ 2D FLCS)", and here we report its first application to the conformational dynamics of a singly-labeled hairpin DNA (Fig.1A). For comparison, we also performed conventional 2D FLCS study on a FRET-pair labeled DNA (Fig.1B).

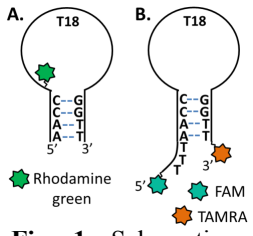

Fig. 1. Schematic of (A) singly labeled and (B) FRET-pair labeled hairpin DNA.

[Methods] 2D FLCS measurements were performed on 5 nM DNA samples at pH 8 (10mM TRIS-HCl), following a protocol described earlier [1]. 200 mM NaI was used for dynamic quenching experiments. For the control experiments, 200 mM NaCl was used instead of NaI. Individual lifetime distributions and 2D lifetime correlation maps were obtained from the global 2D MEM analyses on 2D emission delay maps as explained in [1].

[Results and Discussion] The 2D FLCS measurement on the singly labeled DNA in the absence of a quencher yields a single lifetime distribution (Fig. 2A). In contrast, two lifetime distributions separate out upon addition of the quencher (200 mM I^-, Fig. 2B). Fig. 2D shows the fluorescence decays calculated from the two lifetime distributions as well as that obtained without the quencher. It is clear that the dye is dynamically quenched in both the states, although the average fluorescence lifetime, i.e. the degree of quenching, is significantly different. Since it is expected that the open state of the hairpin DNA is more solvent exposed than the folded state, we assign the shorter lifetime distribution (red distribution in Fig. 2B) to the open state and the longer one (blue distribution) to the folded state. The multi-peak feature in these lifetime distributions possibly arises from sub-µs local dynamics within each state.

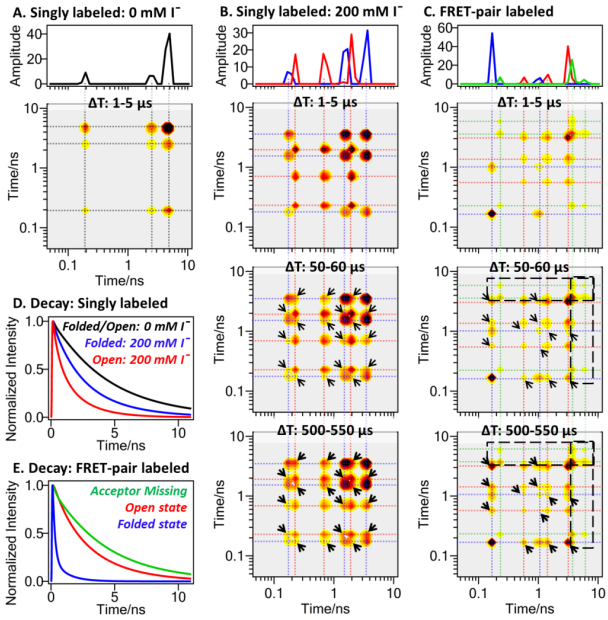

Fig. 2. (A-C) Lifetime distributions of distinguishable species (top panels) and corresponding 2D lifetime correlation maps at specified delay times (bottom panels) for singly labeled DNA without iodide (A) and with 200 mM iodide (B), and for FRET pair labeled DNA (C). The cross-peaks between the blue and red distributions in (B, C) are marked with black arrows. The cross-peaks of the green distribution with red/blue distributions in (C) are expected within the black broken boxes. **(D, E)** Fluorescence decays of individual states corresponding to the lifetime distributions in A-C are shown in corresponding colors for singly labeled DNA (D) and for FRET-pair labeled DNA (E).

The appearance of cross-peaks between the open and folded states is seen in the 2D lifetime correlation map at ΔT=50-60 µs (Fig.2B, marked with black arrows). It indicates the reaction between these two states. Further intensification of the same cross-peaks indicates the progress of reaction at ΔT=500-550 µs. A quantitative analysis of the cross-peak intensity suggests ~50-100 µs timescale for this dynamics.

The 2D FLCS measurement on the FRET-pair labeled DNA yields three lifetime distributions (Fig 2C; corresponding fluorescence decays are shown in Fig. 2E). We assign the shortest lifetime distribution (blue distribution) to the folded state, because a short dye-pair distance in the folded state should result in high FRET efficiency and a short donor fluorescence lifetime. The red distribution, with longer fluorescence lifetime corresponding to a longer dye-pair distance, is assigned to the open state. These two states show cross-peaks at ΔT=50-60 µs (Fig 2C, black arrows), which further intensify at ΔT=500-550 µs. This dynamics is similar to that observed from DQ 2D FLCS. Note that the third state (green distribution) is assignable to acceptor-missing molecules, because it has the longest fluorescence lifetime and shows no cross-peaks with other two states as indicated with the black broken boxes in Fig. 2C.

In summary, we developed DQ 2D FLCS method which can investigate µs-resolved dynamics of local solvent accessibility of a singly dye-labeled biopolymer. The application to hairpin DNA showed a consistent result with the FRET-based 2D FLCS measurement. The information about the site-specific solvent exposure obtainable from DQ 2D FLCS shed new light on the dynamics of biopolymers and is complementary to the dye-pair distance information obtained from FRET measurements.

[References]

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