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# Probing the Structural Distribution and Opening-Closing Rate of Hairpin RNA by 2D Fluorescence Lifetime Correlation Spectroscopy

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## Introduction

The structural dynamics of RNA contains the information about fundamental biological processes that regulate unique biological functions. Characterization of the nature and time scale of these processes facilitates our understanding of the role of RNA in biological systems. However, the elucidation of complicated structural dynamics of RNA is still difficult. In this work, we used a novel 2D fluorescence lifetime correlation spectroscopy (2D FLCS)[1] to investigate a FRET dye-labeled hairpin RNA. By comparing with a DNA analogue, we realized the origins of opening-closing transition of RNA accurately.

## Experiment

In this experiment, we measured the hairpin RNA (UUUAACC$(U)_{18}$GGUU) labeled with a FRET dye-pair 6-FAM and TAMRA as the donor and acceptor, respectively. The DNA analogue was designed with the same sequence to RNA (U was replaced by T in the DNA sample). They were prepared in a buffer solution (10 mM Tris-HCl, 1 mM EDTA, BSA 0.01 %, pH 8.0) with 0.4 M NaCl. Two-color 2D FLCS was performed by a home-built system.[1,2] Each collected photon contains two temporal information: the absolute arrival time determined from the start of experiment ($T$) and the relative arrival time determined from the excitation laser pulse ($t$). The 2D emission-delay correlation map $M(\Delta T; t', t'')$ was created at a characteristic time interval ($\Delta T$) between two photons emitted at $t'$ and $t''$ from the excitation. The maximum entropy method (MEM) was utilized to convert 2D emission-delay correlation maps to 2D fluorescence lifetime correlation maps so as to obtain the lifetime distribution and the correlation of independent species. The lifetime distribution, which corresponds to the structural distribution, was utilized to develop the filter functions[3] to obtain the species-specific correlation. The obtained species-

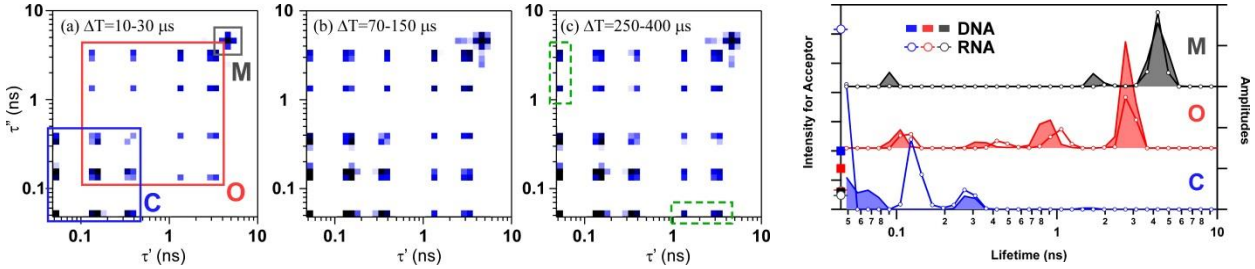


Fig. 1. The 2D fluorescence lifetime correlation maps of RNA at (a) ΔT = 10-30 μs, (b) ΔT = 70-150 μs, and (c) ΔT = 250-400 μs. The solid line squares denote the independent components and dash lines mark the cross peaks. (d) Fluorescence lifetime distributions of RNA and DNA (open symbol: RNA; filled symbol: DNA). The left symbols mean the emission intensity from acceptor dye, which corresponds to the concentration and FRET efficiency of the independent species.

specific correlation is fitted by two-state model to directly extract the equilibrium constant and opening/closing rate constants from FCS photon data.

**Results and Discussion**

Fig. 1(a)-(c) shows the 2D maps of RNA at ΔT = 10-30, 70-150, and 250-400 μs, respectively. The diagonal peaks representing the independent components are assigned as open (**O**), closed (**C**), and acceptor-missing forms (**M**).[1c] Both open and closed forms are composed of several lifetime components, indicative of the structural inhomogeneity. The cross peaks reflect the correlation between open and closed forms, which emerge at ΔT = 70-150 μs and grow to the maximum intensity at ΔT > 250 μs. By contrast, the appearance time and growing time of cross peaks for DNA are ΔT = 30-60 μs and >100 μs, respectively (not shown). It means that the opening-closing transition for RNA is slower than DNA. Fig. 1(d) shows the difference in lifetime distributions between RNA and DNA. The results illustrate the different structural dynamics between RNA and DNA even that they have similar structural distributions.

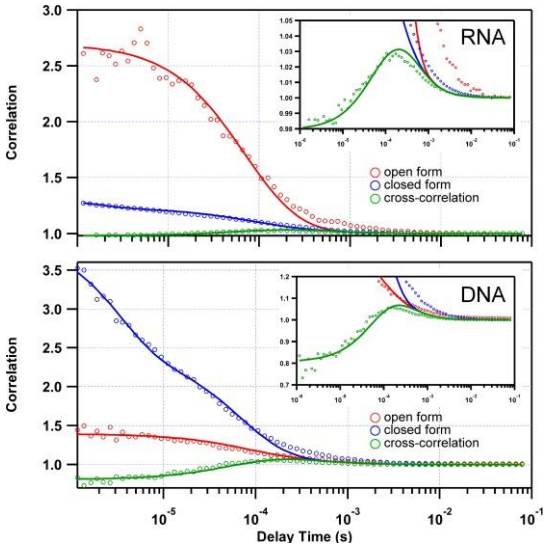

Fig. 2. Species-specific auto- and cross-correlation for open and closed forms of the RNA and DNA samples. The solid lines are the simulation results based on species-specific two-state model. The inset is the zoom-in for the cross correlation curve.

Fig. 2 shows the species-specific auto- and cross-correlation of the open and closed forms of the RNA and DNA samples. The results show that the opening rate constant of RNA is approximately 10 times slower than DNA ($k_{open} \approx 530\ s^{-1}$ and $6050\ s^{-1}$ for RNA and DNA, respectively); in contrast, the difference in closing rate constant is relatively small (that of RNA is roughly twice faster than DNA). The dramatic difference in the opening process is associated to the dissociation of different duplex structures in the stem region, which is A-form for RNA and B-form for DNA.[4] The A-RNA is more tightly wound than B-DNA, causing very slow opening process.

In conclusion, the structural dynamics of hairpin RNA was successfully compared with the DNA analogue using 2D FLCS combined with the filter functions. The opening and closing mechanisms were analyzed in detail, showing that the difference in the opening-closing transition between RNA and DNA is controlled by the opening process rather than the closing process. This study provides new insight into the fundamental biological processes of RNA and DNA.

**Reference**


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