Color-changing fluorescent barcode based on strand displacement reaction for multiplexed imaging of biomolecules

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Keywords: Strand displacement reaction; Fluorescent probes; Nucleic acids nanotechnology; Artificial nucleic acid

Fluorescence imaging has been used in a wide field of biotechnological applications. However, the number of detectable biomolecules is strictly limited, typically to five, due to spectral overlaps of excitation and emission spectra. This limitation prevents the understanding of complex biological functions involving many biomolecules.

In this study, we developed a novel fluorescence labeling method, color-changing fluorescent barcode (CCFB), where fluorescence colors are changed via toehold-mediated strand displacement reaction of nucleic acids (Fig. 1). The CCFB enables the detection of multiple molecules with fluorescence color sequences. The targets are labeled with barcode complex of F strands tethering a fluorophore or a quencher at each terminus. In the initial state, only the terminal fluorophore can emit fluorescence whereas the other fluorophores are quenched by neighboring quenchers. The fluorescence color sequence of barcode can be decoded by the sequential addition of Q strands (Q1, Q2, and Q3) that are fully complementary to F strands. The sequential removal of F strands changes emissive fluorophores in the pre-determined order, allowing simultaneous detection of a large number of targets. When four types of fluorophores are used and fluorescence color is changed three times, $4^{3+1} = 256$ types of molecules can be discriminated. Therefore, this method will allow us to detect an enormous variety of biomolecules simultaneously.

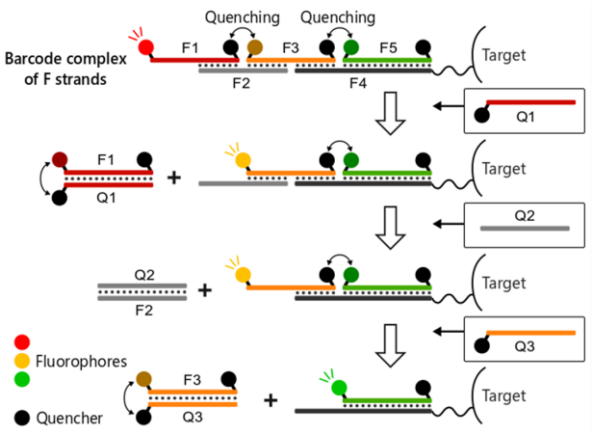

Fig. 1 Schematic illustration of color-changing fluorescent barcode method based on strand displacement reaction.

We synthesized oligonucleotides with three fluorophores Cy5, Cy3, and FAM, as components of fluorescent barcode. When Q strands are added to barcode complex, the intended fluorescence color changes were confirmed by fluorescence measurements. Furthermore, the applicability of our method to immunofluorescence was investigated. Fluorescent barcode was conjugated to antibody through the heterobifunctional crosslinker. Immunostaining with fluorescent barcode-antibody conjugates successfully visualized three proteins in fixed HeLa cells.[1]

1) K. Makino, E. Susaki, M. Endo, H. Asanuma, H. Kashida, *J. Am. Chem. Soc.*, **2022**, *accepted*.