Multiple functional molecules assembled DNA nanostructure for bioimaging application

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Cellular processes are mediated through intertwined complex chemical and physical interactions of biological molecules. Therefore, analytical devices that simultaneously monitor the change of the concentration and the location of biologically important molecules, and the change of cellular environment indicated by various physical parameters will give a comprehensive understanding of molecular mechanisms underlying various cellular processes.

Compared to the conventional sensors comprised of a single sensing molecule, assembling several kinds of fluorescent sensors on a DNA scaffold has advantages in monitoring different targets at the same time within the same nanospace by combining individual functions of fluorescent sensors. A single DNA scaffold allows the assembly of multiple fluorophores, with different responses towards such as pH changes and enzyme activities and so on, to be localized in a defined space, enabling multiple parameter detection.

Firstly, we have developed a ratiometric pH sensor by constructing a DNA nanostructure[1] labeled by two types of fluorophores. The combination of a pH-sensitive fluorophore fluorescein (CF) with pKa of 6.5 and tetramethylrhodamine (CR), a pH-insensitive fluorophore under the physiological conditions, assembled on DNA scaffold had resulted in a reliable ratiometric pH detection in a test tube. The sensor was successfully applied for real-time sensing of cellular pH changes throughout its internalization by its fluorescence signal variation under microscopic conditions.[2] Next, the detection range was expanded by coassembling with Oregon Green (OG) (pKa = 4.8). We also applied protease activity detectable fluorescent probe[3] on the DNA scaffold to detect multiple parameters on the same nanospace.

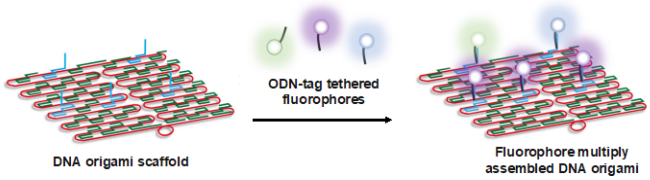

Figure Various fluorescent probes assembled DNA origami scaffold for multiple target monitoring.

References

[1] Rothemund, P. W. K. *Nature*, **2006**, *440*, 297.
[2] Nakata, E.; Hirose, H.; Gerelbaatar, K.; Arafiles, J. V. V.; Zhang, Z. Futaki, S.; Morii T. *Chem. Sci.*, **2021**, *12*, 8231.
[3] Zhang, Z.; Nakata, E.; Shibano, Y.; Morii, T.*ChemBioChem*, **2022**, *23*, e202200319.

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