NANOBIOTECHNOLOGY

Dye shines bright

A tiny lightning rod made of two gold particles and a DNA pillar creates a hotspot that brightens fluorescent signals in zeptoliter volumes.

Single molecules are a sight to behold—that is, if you can see them. To catch a glimpse of a DNA strand adding a base, fluorescent dyes must shine consistently and brightly, but often the signal can get lost in a sea of visual noise. Fluorescence needs a lift.

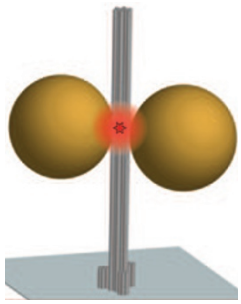
To create a hotspot that delivers a more than 100-fold illumination boost, scientists in the NanoBioSciences group at Braunschweig University of Technology used a nanoantenna made from a pillar of self-assembled DNA and two gold particles.

The nanoantenna can help researchers record signals from single molecules over time. "A typical application would be single-molecule sequencing, by watching a polymerase incorporate single dye-labeled nucleotides into the newly formed strand," says principal investigator Philip Tinnefeld. Besides boosting fluorescence, the team lowered the possible detection volume to a zeptoliter, which is equivalent to 10^{-21} liters.

The nanoantenna that reinforces the light signal is made up of two gold nanoparticles hitched to a 220-nanometer-high pillar, standing steady on a cover slip. The pillar is built using DNA origami folding techniques. "The DNA origami pillar itself is our breadboard, where we can place the two nanoparticles with nanometer precision," says Guillermo Acuna, a postdoctoral fellow in the lab.

The team used the open-source software cadnano (www.cadnano.org/) to design the DNA pillar with attachment sites for the dye and a docking site in what turned out to be the "plasmonic hotspot." DNA origami involves a long strand of phage DNA and short DNA sequences that function as staples, which all self-assemble into predesigned shapes. The technique takes advantage of the way certain nucleotides preferentially pair-bond with one another. By picking the right sequence of bases, researchers predetermine the shape of the object to be built with DNA.

Between the gold particles is around 25 nanometers of berth, which becomes a hotspot, for a single fluorescent dye molecule. The particles form a bond, creating a 'nanolens' that focuses light and enhances the fluorescent signal. Unlike a conventional lens that changes the trajectory of incoming light rays, the gold particles focus the light in the space between them. "It is pretty much like in the lightning rod effect," Acuna says. The light's electric field exerts a force on the free electrons in the metallic nanoparticles, displacing them and polarizing the nanoparticles. The team showed that, when positioned in this nook, the dye molecule delivered a 117-fold higher level of fluorescence.



A hotspot that enhances fluorescence sits in a nook between two gold nanoparticles hooked to a DNA origami pillar. Image courtesy of F. Möller and P. Holzmeister.

The researchers experimented with nanoparticles between 20 and 100 nanometers in diameter, with the bigger ones giving better fluorescence enhancement.

The scientists imaged binding of short DNA strands and, separately, conformational shifts in a DNA Holliday junction, which is formed between four strands of DNA during cell division.

The nanoantenna arrangement is more flexible than most current techniques, "so we can also use the DNA origami pillar to place our fluorophore or other biological assays exactly in the focus of the nanolens," Acuna says.

The scientists believe their method has components familiar to many scientists, which will help others try this approach. "Many groups have worked with DNA origami structures with single fluorescent dyes, and other groups have bound different metallic nano-objects to origamis," says Acuna. The team is now exploring other ways to apply this hotspot and also to optimize the space between the nanoparticles and further improve fluorescence levels.

Vivien Marx

RESEARCH PAPERS

Acuna, G.P. *et al.* Fluorescence enhancement at docking sites of DNA-directed self-assembled nanoantennas. *Science* **338**, 506–510 (2012).