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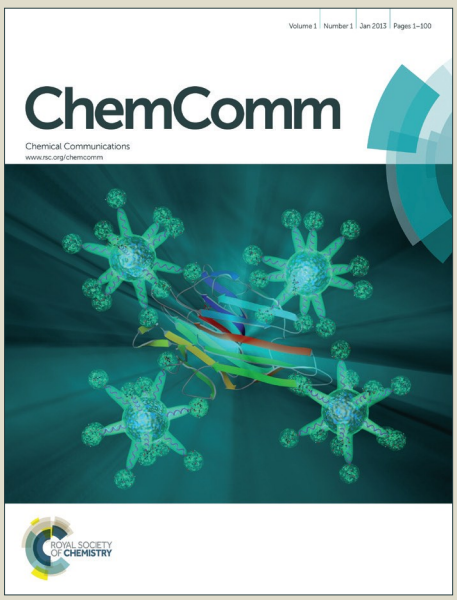

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Nucleic-Acid-Programmed Ag-Nanoclusters as a Generic Platform for Visualization of Latent Fingerprints and Exogenous Substances

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We display a nucleic acid controlled AgNCs platform for latent fingerprints visualization. The versatile emission of aptamer-modified AgNCs were regulated by the nearby DNA regions. Multi-color images for simultaneously visualization fingerprints and exogenous components were successfully obtained. A quantitative detection strategy for exogenous substances in fingerprints was also established.

Fingerprint analysis is an indispensable part of forensic investigations, access control, and medical diagnostics today, since the fingerprint is regarded as important physical personal identification.[1] The fingerprint is composed of secretions, skin oils and other components in sweat. [2] On the other hand, it not only consists of perspiration and natural secretion residues, but also contains exogenous compounds from the environment.[2] Therefore, the imaging of fingerprint has excited intense interests in the fields of identifying drugs, residues of explosives, and other secreted chemicals.[3] An impression of finger without blood or paint is known as a latent fingerprint (LFP) for its invisibility to the bare eye. In order to visualize LFPs, researchers often generate an optical contrast between the ridges of the fingerprint and the affected surface.[4] There have been several methods for detecting fingerprints or enhanced LFP imaging such as fluorescence imaging, electrochemiluminescence, scanning electrochemical microscopy and dark-field microscopy.[5] Among these existing methods, fluorescence imaging is the most common used approach with high sensitivity.[6] For example, recently, Yuan and coworkers reported the use of DNA conjugated upconversion nanoparticles as luminescence materials for LFP imaging.[7] This approach can be applied to fingerprints on different surfaces and from different people under near-infrared-red light. However, these reported strategies often focus on taking the images of LFPs and the exogenous components independently. Moreover, when detecting variety of compounds, the probes have to be synthesized or functionalized again. On the other hand, the amount of components in LFPs is an important parameter in some fields such as medical diagnostics. Nevertheless, the detection of the substances by using the above approaches is always only imaging, which is qualitative rather than quantitative. Hence, it is of great importance to developing a facile approach for imaging of LFPs with exogenous components and quantitatively recognizing those components in contact with a fingertip.

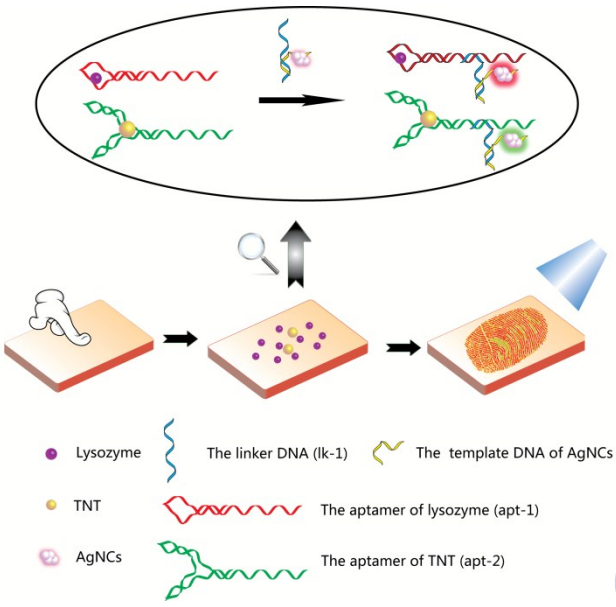

Fig. 1 Schematic representation for visualizing the LFPs using DNA-regulated AgNCs. The Lysozyme and TNT in LFPs was bond by different aptamers. The AgNCs were placed in close proximity to the different regions of the aptamers by the linker DNA, causing multi-emitting of the LFPs.

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DNA-Ag nanoclusters (DNA-AgNCs) have received much attention over the past decade.[8] They circumvent many of the shortcomings of conventional molecule beacons and offer excellent potential in cellular imaging, information possessing, and chemical/biological detection.[9] DNA-AgNCs are promising as fluorescent reporters, since the fluorescence properties of the AgNCs are dependent on the microenvironments provided by the nearby DNA chain.[10] Werner and co-workers demonstrated that some dark AgNCs could be converted into bright-red/green emitters when in close proximity to G-rich or T-rich DNA, which could be successfully applied to DNA sensing.[11] This fact offers an easy way to detect multiple analytes in a complex system by using appropriate DNA molecule with AgNCs. Compared with other fluorescent probes, AgNCs shows great potential for LFP imaging owing to their ease of synthesis, outstanding photostability, excellent biocompatibility and easily-modulated fluorescence.[12] In addition, considering the wide availability of the DNA aptamers, the AgNCs-based fluorescent probe can be employed as an exceptionally versatile and generic platform for identifying any aptamer-bound molecules.[13] Thus, we believe that the DNA-AgNCs could provide a general and simply performed platform for visualization of components in latent fingerprints as well as quantitative detection of exogenous components.

Herein, for the first time, we employed multi-color C-rich DNA templated AgNCs for simultaneously imaging of those exogenous components in LFPs and the LFPs themselves. We chose Lysozyme as the model target for LFP imaging in this work. In order to bind to the ridges of the fingerprint, as shown in Fig. 1, a DNA aptamer (apt-1) was utilized for binding Lysozyme while a linker DNA (lk-1) was chosen to partly hybridize with the extensive strand of the apt-1.[14] Moreover, a G-rich region was designed at the end of the extensive part of apt-1. Upon the addition of the DNA (mb-1) templated AgNCs (mb-1-AgNCs), the three DNA strands could assemble to a structure similar to a 3-way junction. Since the hybridization placed the AgNCs in close proximity to the G-rich region of apt-1, the dim AgNCs could be lighted up into bright red-emitting AgNCs. Thereby, the fluorescent image of the ridges of LFPs with red color could be caught by naked eyes under irradiation. Most importantly, this approach enabled us to identify variety of compounds by just changing the sequence of the apt-1 without any extra functionalization. We further investigated the detection of explosive residues left on fingertips as an example of exogenous substances by using this strategy. Another DNA aptamer (apt-2) was chosen as a general targeting reagent for binding trinitrotoluene (TNT), one of the most common used explosive.[15] A T-rich region was included in the extensive strand of apt-2. This T-rich region could modulate the fluorescence of the AgNCs in mb-1 and convert them into green-emitting fluorophores. In such way, a versatile image of a LFP with TNT could be recorded in which the red region represented the fingerprint and the green region indicated the existence of the TNT molecule. Based on the multi-color emission of the AgNCs, a ratiometric strategy for quantitatively recognizing exogenous substances could be established.

In the experiment, the DNA-AgNCs were synthesized using reported method.[11] Briefly, the AgNCs are created through direct reduction of Ag salts by BH_4^- ions within ssDNA scaffold. The obtained DNA-AgNCs were characterized by the transmission electron microscope (TEM). As shown in Fig. S1, the mean size of the AgNCs was 2nm, which was consistent with the literature.[16] The fluorescent features of the DNA-AgNCs were demonstrated in Fig. S2. The solution of mb-1-AgNCs showed dim red emission. Upon addition of lk-1 and apt-1, a strong fluorescence emission (610 nm) was observed from the solution under the excitation with 580nm. On the other hand, when added lk-1 and apt-2, the AgNCs were transformed into bright-green-emitting clusters with an emission wavelength of 530 nm when exited at 480 nm. Due to the DNA scaffolds, the AgNCs exhibited excellent water dispersibility with luminescence red and green in color under excitation, respectively (Fig. S3). Furthermore, no obvious fluorescent change of AgNCs occurred in the aqueous after kept in 4 ℃ for 2 weeks (Fig. S4).

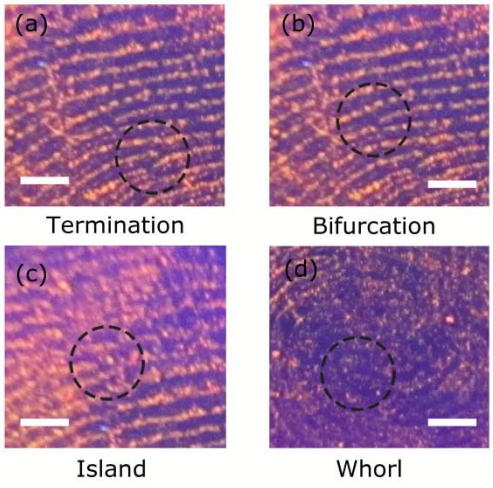

Fig. 2 (a) The detail structure of a latent fingerprint visualized by red emitting AgNCs. All the scale bars corresponded to 2 mm.

The feasibility of our strategy to image LFPs was first confirmed using a UV transillustrator. Fig. S5 showed the images of LFPs imaged with AgNCs on a quartz chip. Fingerprints only treated with mb-1-AgNCs did not show fluorescent images (Fig. S5). This indicated that the fingerprints were not bound by the AgNCs without the aptamer and the linker DNA. Then we pretreated the LFPs on the quartz chip with lk-1 and apt-1. Upon the addition of mb-1-AgNCs, the lk-1 played a role of bringing the AgNCs and the G-rich region into proximity. The hybridization results were supported by a gel electrophoresis experiment of mb-1, lk-1 and apt-1 (Fig. S6). The observed bands corresponded to mb-1 (lane 1), apt-1 (lane 2), lk-1 (lane 3) and the hybridization product (lane 5), respectively. As a result, we obtained clear luminescence images on the chip, which could be visualized by naked eyes (Fig. S7). The details of the fingerprints, such as terminations or bifurcations were also clear observed (Fig. 2).

In order to obtain a higher magnification image, we made use of the fluorescence microscopy to catch the details of the LFPs. Fig. 3 showed the emission images of fingerprint under bright field and the AgNCs labeled lysozyme under excitation. It is easy to see that the fluorescent area overlapped on the fingerprints area perfectly, indicating that the fluorescent image could represent the shape and location of the LFP. Thus the results proved the excellent performance of our strategy for LFP imaging.

We then used the DNA-AgNCs to identify TNT in the fingerprints. A certain concentrations of 100 μL TNT solutions were dropped on the fingers. The fingerprints of these fingers were collected after the solutions were dried in air. We pretreated LFPs with lk-1 and the apt-2 instead of the apt-1. The AgNCs and the T-rich region were brought into proximity and converted into green-emitting clusters (Fig. S8a). The hybridization results were also supported by the gel electrophoresis experiment. The DNA mb-1 (lane 1), lk-1 (lane 3), apt-2 (lane 4) and the hybridization product (lane 6) were showed on the gel, respectively (Fig. S6). As expected, the LFPs exhibited a green luminescence image on the chip under UV irradiation (Fig. S8b). When came to the images recorded by fluorescence microscopy, the fluorescent areas (represented TNT areas) were all placed inside the fingerprint areas (Fig. S8c).

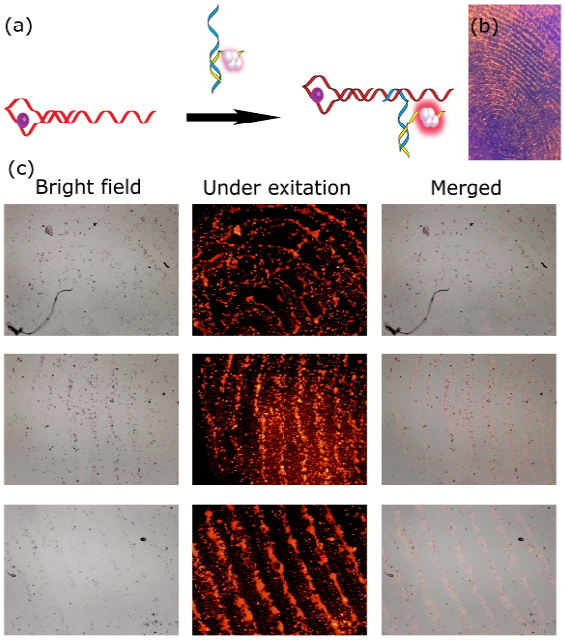

Fig. 3 (a) Principle of the visualization of the LFPs by the lightened-up AgNCs. (b) Fluorescent image on a quartz chip under irradiation (c) Higher magnification fluorescent images captured by fluorescence microscopy

We further investigated the capability of simultaneously imaging the LFPs and the exogenous component by using the same bioprobe. All the three ssDNA apt-1, apt-2 and lk-1 were pre-incubated with LFPs and the similar experiments were carried out on the fingerprints (Fig. 4a). The resulting image was exhibited in Fig. 4b. Different from the above two cases, the images showed two different luminescent colors which represented two components in the fingerprint. The LFP was lightened up by the red emission of the AgNCs while the TNT was high-lighted by the same bioprobe, which emitting green luminescence. On the other hand, three different concentrations of TNT solutions were doped in the LFPs, and high magnification images were captured under two different excitations and the bright field (Fig. 4c). The labeled lysozyme and TNT were separated into two channels of red and green, suppressing the interference from each other. With the decrease of the TNT loading, we observed different brightness of green fluorescence, indicating the variety of the green-emitting AgNCs. Fingerprint doped with 100 μg TNT demonstrated a high-light green emission. When decreasing the doped amount of TNT to 10 μg, it is obviously that the green emission was weakened but still visible, compared with the red part. Nevertheless, it is hardly to visualize the ridge image of the green channel with 1 μg of the doped amount of TNT. These results supported that out strategy provided a simple way for visualization and locating of two different components in LFPs at once. It is worth noting that the main AgNCs used in all these experiments are unchanged. In addition, in this approach, the apt-1 and apt-2 can be replaced flexible by other aptamers for identification of other molecules without changing the sequence of the DNA template and recreating new clusters.

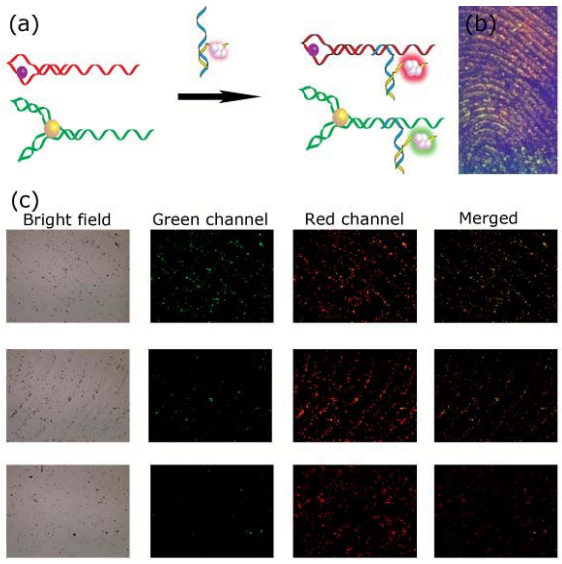

Fig. 4 (a) Principle of simultaneously visualizing of the LFPs and TNTs by the lightened-up AgNCs. (b) Fluorescent image on a quartz chip under irradiation (c) Higher magnification fluorescent images captured by fluorescence microscopy. The concentrations of TNT were 100 μg, 10 μg and 1 μg from the top row to the bottom, respectively.

According to the above results, since the fluorescent intensity of the green emission was related to the concentration of TNT, it is promising that the dual-emission of the AgNCs could be applied as a probe for ratiometric detection of substances in fingerprints. The quartz chips with treated fingerprints were erected on the sample holder of a fluorescence spectrometer, and the intensity of the AgNC-labeled compound in LFPs could be recorded. Fig. S9 showed the fluorescent (FL) spectra excited in 480 nm of the LFPs pretreated with apt-1 and apt-2, respectively. Compared with

the FL spectra in solution, the peak position of the red-emitting AgNCs shifted a little. This different may arise from the differences of excitation wavelength or the interaction with the compound in fingerprints. In the FL spectrum of a dual-emitting LFP, one emission peak at 573 nm represented the amount of lysozyme acting as an internal reference and enhancing the signal-to-noise ratio, and the other at 531 nm corresponded to the TNT concentrations (Fig. S10). Fig. S10d presents the fluorescence data as a function of ratiometric fluorescent intensity versus the amount of doped TNT (10 μL solution). The fluorescence intensities were proportional to the TNT concentration in the range from 0.1 to 10 μg, and the detection limit of TNT was calculated as 54.7 ng. This result demonstrated that this design could provide a quantitative identification of components in LFPs. Therefore, this strategy can be successfully applied to multiple detection of chemical makeup in fingerprint and would show great potential in the field such as drug detection and medical diagnostics.

Conclusions

In summary, we have demonstrated a facile and general strategy to visualize and detect latent-fingerprint by combining the DNA-modulated AgNCs with the molecule-binding aptamers. This method is simple in operation and cheap in cost. The emissions of the AgNCs are regulated by the nearby DNA region, resulting in a multi-color image of LFPs. Clear images can be visualized under excitation by naked eyes and the detail of the LFPs can be directly recorded by fluorescent microscopy. In addition, the emission-modulating region and the aptamer region can be combined together at will, thus providing a simultaneous identification for multiple components. Moreover, the AgNCs do not need to be re-created or be further functionalized, even if the analytes are changed. In consideration of the diversity of aptamers, our strategy is able to be developed to a generic platform and potentially extended to analyzing variety of aptamer-binding molecules in fingerprint. Most importantly, a quantitative method for recognizing components in LFPs has been established in our works, which promises the approach to widely apply in diagnostics. Therefore, this proof-of-concept may open a new door to for LFPs identification in future studies.

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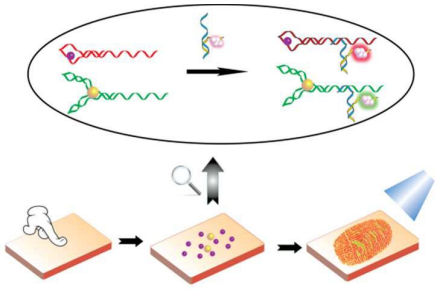

A nucleic acid controlled AgNCs platform for simultaneously imaging and quantitative detection of substances in fingerprints.