

# Aptamer-conjugated nanomaterials for bioanalysis and biotechnology applications

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In recent years, nanomaterials have captured the attention of scientists from a wide spectrum of domains. With their unique properties, nanomaterials offer great promise for numerous applications, ranging from catalysis to energy harvesting and information technology. Functionalized with the desired biomolecules, nanomaterials can also be utilized for many biomedical applications. This paper summarizes recent achievements in the use of aptamer-conjugated nanomaterials for bioanalysis and biotechnology applications. First, we discuss the features and properties of aptamers and then illustrate the use of aptamer-conjugated nanomaterials as sensing platforms and delivery vehicles, emphasizing how such integration can result in enhanced sensitivity and selectivity.

## Introduction

Nanomaterials are structures having a size of 100 nanometers or smaller in at least one dimension. Because of quantum effects stemming from the large surface area to volume ratio, nanomaterials possess unique optical,<sup>1</sup> electronic,<sup>2</sup> magnetic,<sup>3</sup> mechanical,<sup>4</sup> physical and chemical properties.<sup>5</sup> Thus far, nanomaterials have generated widespread interest and applications in energy harvesting<sup>6</sup> and information technology.<sup>7</sup> In energy harvesting, nanomaterials are commonly applied to reduce energy consumption, improve energy production efficiency, and exploit energy systems compatible with the environment. Meanwhile, information technologists are mainly concerned with the use of nanomaterials for memory storage expansion, novel semiconductor development and optoelectronic device

construction. Recently, however, investigators have prepared nanomaterials for use in biomedicine, where increasing applications have been found in biosensor development,<sup>8</sup> molecular imaging,<sup>9</sup> drug delivery<sup>10</sup> and cancer therapy.<sup>11</sup>

Both enhanced biocompatibility and biofunctionality can be achieved by conjugating nanomaterials to synthetic (*e.g.*, polyethylene glycol (PEG)) or natural (*e.g.*, folic acid) ligands.<sup>12</sup> In particular, aptamers have found increasing applications in such bioconjugated systems. Aptamers are single-stranded oligonucleotides generated from an *in vitro* process known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment).<sup>13</sup> By folding into distinct secondary or tertiary structures, aptamers can specifically bind to their targets with dissociation constants in the micromolar to picomolar range. Targets range in scope from small molecules<sup>14</sup> to proteins,<sup>15</sup> whole cells<sup>16</sup> and even tissues.<sup>17</sup> Our lab has developed a cell-based SELEX strategy that can produce a panel of aptamers for diseased cells, especially cancer cells.<sup>18</sup> Some aptamers can be internalized into living cells,<sup>19</sup> thus making them good candidates for drug delivery.

Because of the molecular recognition property of aptamers, they are sometimes called chemical antibodies. However,

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compared to antibodies, aptamers possess several unique advantages. First, the *in vitro* selection process of aptamers eliminates the need for the *in vivo* immunization of animals to generate antibodies, thus greatly widening the range of targets for aptamers, to include toxic or non-immunogenic materials.<sup>20</sup> Second, aptamers can be easily prepared *via* a solid-phase oligonucleotide synthesis with good reproducibility.<sup>21</sup> Compared to antibodies, aptamers are much more stable to heat, pH and organic solvents.<sup>22</sup> Third, the chemical modification of aptamers with organic fluorophores, redox labels, functional groups or even nanomaterials is relatively simple.<sup>23</sup> Finally, other than the specific molecular recognition toward their targets, aptamers are

capable of recognition through base-pairing, enabling tailorable designs for desired applications.<sup>24</sup>

For a fair comparison, however, the disadvantages of aptamers should also be mentioned. One problem is that aptamers are vulnerable to nuclease digestion in cells or biological fluids, such as sera, blood and urine.<sup>25</sup> In addition, some aptamers have relatively weak binding affinities to their targets.<sup>26</sup> Thus, the question becomes whether these disadvantages can be successfully overcome. Regarding the stability issue, modification of aptamers with 2'-aminopyrimidine has generated anti-bFGF aptamer that can survive in urine for up to 17 h.<sup>27</sup> In addition, modification of aptamers with 2'-fluoropyrimidine<sup>28</sup> or



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2'-O-methyl nucleotides<sup>29</sup> has also achieved similar success. As for weak binding, many molecular engineering strategies have greatly improved the affinities of aptamers toward their targets.<sup>30</sup>

On balance, the myriad of nanomaterials available for the construction of aptamer-based bioconjugates will still result in structures capable of precise molecular recognition and enhanced target specificity according to pre-defined biochemical and biomedical applications. To demonstrate this point, the synthesis and properties of various nanomaterials, such as silica nanoparticles, gold nanoparticles, carbon nanotubes, gold nanorods, liposomes and micelles, are reviewed. Then the integration of these nanomaterials with aptamers is discussed. Finally the applications of these aptamer-conjugated systems as sensing platforms for bioanalysis and delivery vehicles are given.

### Aptamer-conjugated nanomaterials as sensing platforms for bioanalysis

Understanding basic biological phenomena with the aim of exploring potential therapeutic applications depends on the identification and characterization of biomolecules and biological interactions. To achieve these objectives, suitable biosensing platforms are required. Two essential characteristics of biosensors are molecular recognition and signal transduction. Nanomaterials add signal transduction, as well as signal amplification. Meanwhile, aptamers have excellent molecular recognition ability. Thus, the integration of nanomaterials and aptamers will lead to a potentially promising construct for biosensing platforms. Today, rapid development in nanotechnology has resulted in the successful synthesis of numerous nanomaterials for bioanalysis. We will focus on silica nanoparticles, gold nanoparticles, carbon nanotubes and gold nanorods.

#### Silica nanoparticles

Silica nanoparticles, especially dye-doped silica nanoparticles, are currently used in many areas of bioanalysis. There are two standard methods for synthesizing dye-doped silica nanoparticles: the Stöber method<sup>31</sup> and the reverse micro-emulsion method.<sup>32</sup> The former method is relatively simple, and either organic or inorganic dyes can be incorporated. However, it produces dye-doped silica nanoparticles with a relatively large size distribution because of the hydrolysis procedure. The latter method can generate uniform dye-doped silica nanoparticles, but only inorganic dyes can be incorporated. Modifications and optimizations have been applied to make both methods easier and more universal. Apart from single-dye doping, multiple dyes have also been incorporated into the silica matrix.<sup>33</sup>

Compared to common fluorophores, dye-doped silica nanoparticles are superior in three respects:<sup>34</sup> (1) Dye molecules doped inside are immune to degradation and photo-bleaching due to the protection of the silica matrix; (2) No further signal amplification scheme is needed, since thousands of dye molecules are trapped inside one single nanoparticle; (3) Various biomolecules can be introduced onto the surface of a single nanoparticle by the versatility of silicon chemistry. These advantages make dye-doped silica nanoparticles increasingly attractive for use in sensitive bioanalysis.

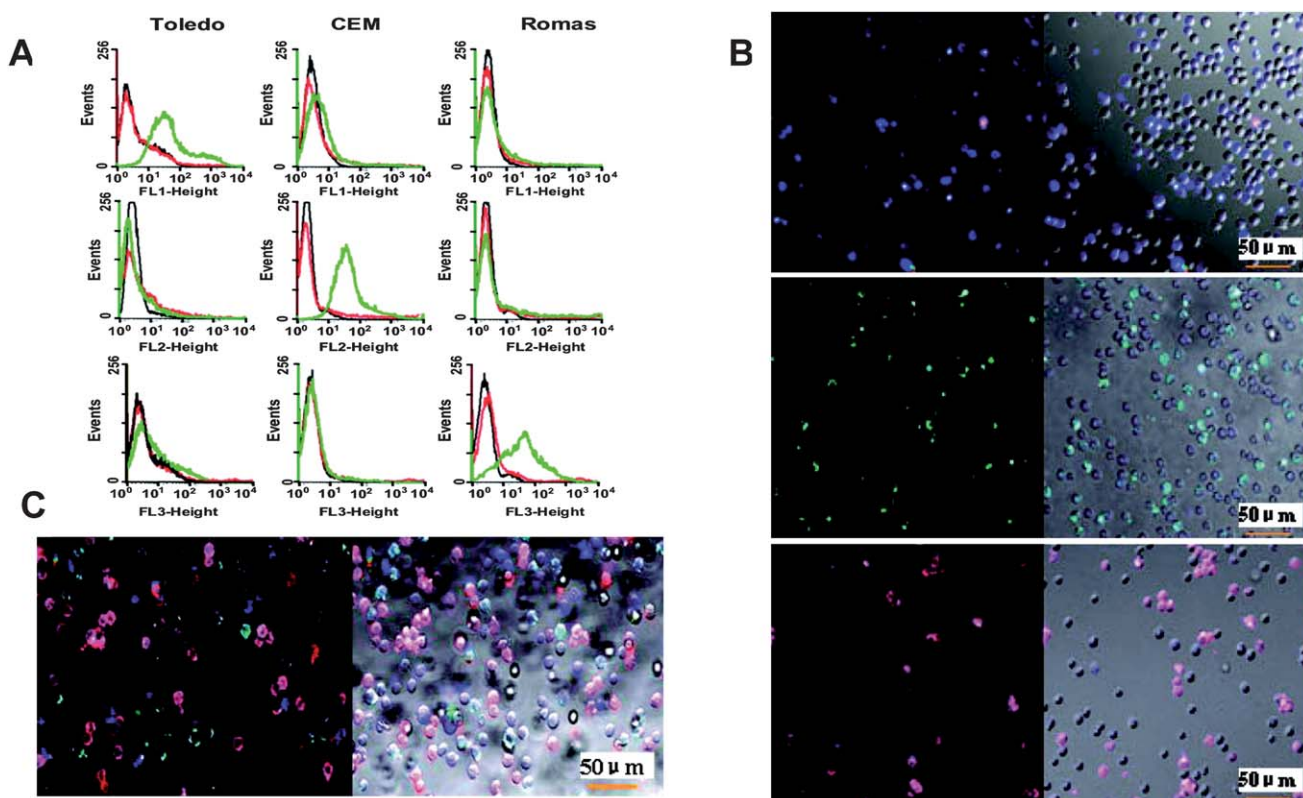
Using dye-doped silica nanoparticles as reporters, many detection methods have been developed for small molecules<sup>35</sup> and proteins.<sup>36</sup> In recent work by Wang *et al.*, a sensitive and selective aptasensor was developed using Ru(bpy)<sub>3</sub><sup>2+</sup>-doped silica nanoparticles as DNA tags for the detection of thrombin, based on target-induced strand displacement.<sup>36</sup> As a consequence of aptamers' high binding affinity and excellent binding specificity, they rival antibodies for molecular recognition. This makes aptamer-conjugated dye-doped silica nanoparticles increasingly used in the area of early diagnostics. For instance, sensitive detection of CEM cells by Sgc8 conjugated dye-doped silica nanoparticles has been achieved.<sup>37</sup> Inspired by the successful synthesis of dye-doped silica nanoparticles with three different types of dyes with efficient fluorescence resonance energy transfer (FRET),<sup>38</sup> Chen *et al.* realized multiplexed cancer cell detection by conjugating this triad of dye-doped silica nanoparticles with three different aptamers specific for three individual cancer cell types [Fig. 1].<sup>39</sup> FAM-doped silica nanoparticles were conjugated with T1 aptamer for Toledo cells; FAM- and R6G-doped silica nanoparticles were conjugated with Sgc8 aptamer for CEM cells; and FAM-, R6G-, and ROX-doped silica nanoparticles were conjugated with Td05 aptamer for Ramos cells. Both flow cytometry histograms and confocal microscopy images showed the selectivity of this assay, demonstrating the high specificity of using aptamers for molecular recognition. Because of the FRET phenomenon between FAM, R6G and ROX dyes, different emissions can be produced by only one single excitation. When compared to single dye-doped silica nanoparticles, these quantum dot-like, multiple dye-doped silica nanoparticles greatly simplify the instrumental setup for multiplexed bioanalysis, since only one laser source is needed.

Dye-doped silica nanoparticles can greatly increase measurement sensitivity because of their inherent signal amplification. Estévez *et al.* have demonstrated this point by using aptamer-conjugated dye-doped silica nanoparticles for cancer cell monitoring on a flow cytometer [Fig. 2].<sup>37</sup> Enhanced sensitivity arises from (1) thousands of dye molecules trapped inside one single nanoparticle and (2) millions of cell membrane receptors for aptamer binding on one cancer cell. In order to further increase sensitivity and simplify sample preparation, magnetic nanoparticles have been introduced into the dye-doped silica nanoparticle based bioanalysis.<sup>40,41</sup> Smith *et al.* developed a novel two-nanoparticle assay for the rapid collection and detection of leukemia cells.<sup>40</sup> In this assay, aptamer-conjugated magnetic nanoparticles were used to simplify sample preparation and realize target cell extraction from complex mixtures, including whole blood samples. Aptamer-conjugated dye-doped silica nanoparticles were used to signal enriched cancer cells. As a consequence of magnetic enrichment, as well as the high affinity and specificity of aptamers, this assay is highly sensitive and selective. Later, by employing a similar principle, Smith *et al.* further expanded this assay for multiplexed cancer cell monitoring.<sup>41</sup>

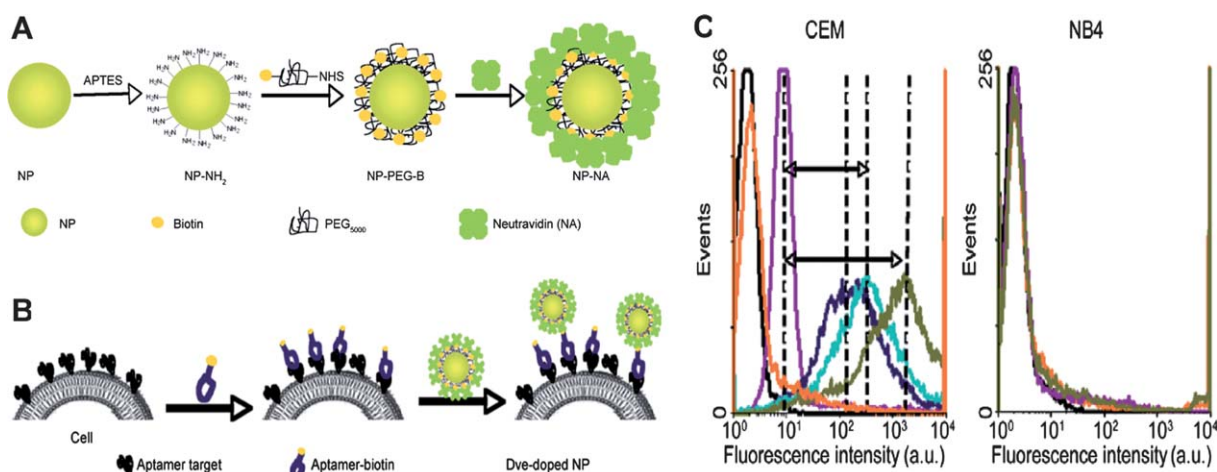
#### Gold nanoparticles

Gold nanoparticles, commonly known as gold colloid or colloidal gold, are also widely used for bioanalysis.<sup>42</sup> Generally, they are produced in a liquid by reduction of chloroauric acid





**Fig. 1** (A) Flow cytometry histograms of aptamer-conjugated dye-doped silica nanoparticles with three different cells: Top row, T1 aptamer conjugated to FAM-doped particles; middle row, Sgc8 aptamer conjugated to FAM- and R6G-doped particles; bottom row, Td05 aptamer conjugated to FAM-, R6G- and ROX-doped particles. On each histogram, the black curve corresponds to cells only; red curve corresponds to dye-doped silica nanoparticles with cells; and green curve corresponds to aptamer-conjugated dye-doped silica nanoparticles with cells. (B) Confocal microscopy images of aptamer-conjugated dye-doped silica nanoparticles with a mixture of three different cells: Top row, T1 aptamer conjugated to FAM-doped particles; middle row, Sgc8 aptamer conjugated to FAM- and R6G-doped particles; bottom row, Td05 aptamer conjugated to R6G- and ROX-doped particles. (C) Confocal microscopy images of all the aptamer-conjugated dye-doped silica nanoparticles with a mixture of three different cells. Adapted from ref. 39.



**Fig. 2** (A) Surface modification scheme for dye-doped silica nanoparticles. (B) Incubation procedures of aptamer-conjugated dye-doped silica nanoparticles with cells. (C) Flow cytometry histograms of increasing amounts of aptamer-conjugated dye-doped silica nanoparticles with CEM cells (target cells) and NB4 cells (control cells). Adapted from ref. 37.

( $\text{HAuCl}_4$ ) and their size can be easily controlled according to specific applications.<sup>43</sup> Standard gold-thiol chemistry allows a monolayer of biomolecules having thiol end groups to be anchored on the gold nanoparticle surface through a ligand

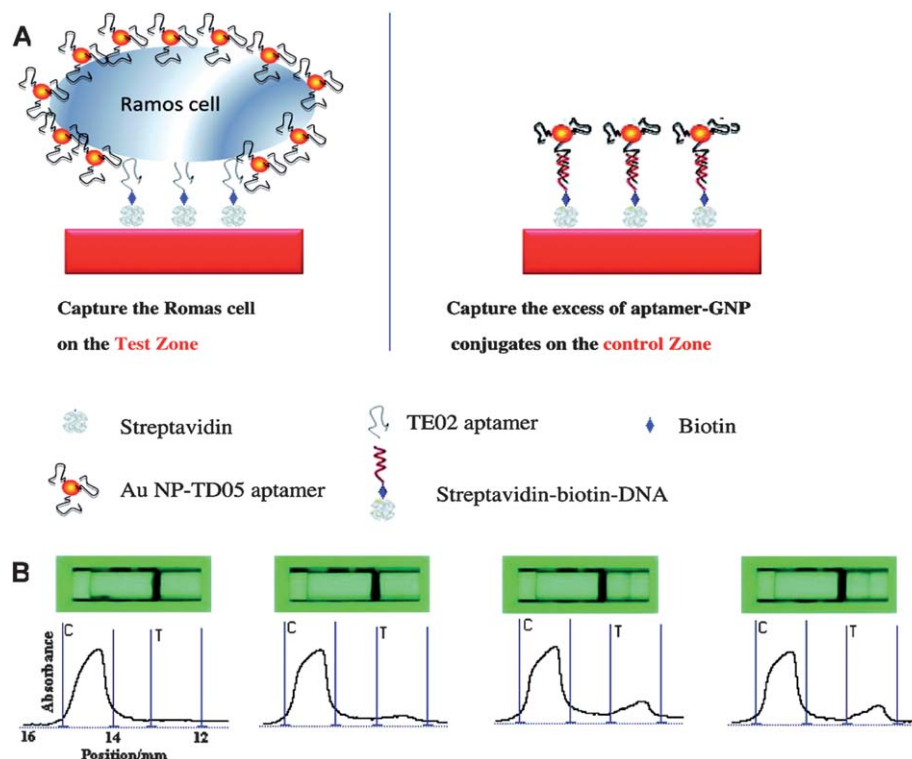
exchange process. As a consequence, combination of gold nanoparticles with thiol bearing ligands (*e.g.*, small molecules,<sup>44</sup> proteins<sup>45</sup> and DNA strands<sup>46</sup>) gives rise to a large number of functionalized gold nanoparticles with different sizes, different

recognition moieties and inherent polyvalent abilities, satisfying the need for a wide range of biological applications.

Due to the spatial length scale reduction of electronic motion and the coherent oscillation of the conduction band electrons, gold nanoparticles possess a strong surface plasmon resonance (SPR) band.<sup>47</sup> In addition, the SPR band of gold nanoparticles has strong distance-dependent properties.<sup>48</sup> That is, when gold nanoparticles come into close proximity with each other, their absorption spectra and scattering profiles change correspondingly, producing a color change in the sample that can be observed by the naked eye. Taking advantage of this phenomenon, a novel method of using aptamer-conjugated gold nanoparticles for colorimetric detection of cancer cells was developed.<sup>49,50</sup> In this assay, gold nanoparticles were assembled and brought together *via* the recognition between aptamers and their target proteins on the cell membrane. This, in turn, caused the SPR bands of the gold nanoparticles to overlap, providing a direct visualization of CEM cells by using Sgc8-conjugated gold nanoparticles. Although slight non-specific binding was observed, this assay was sufficiently sensitive and selective. Experiments on Ramos cells with Td05-conjugated gold nanoparticles showed that this method could also be used for visualizing Ramos cells.<sup>49</sup> Adapting a similar scheme, Liu *et al.* fabricated a strip biosensor for sensitive detection of circulating tumor cells, using Ramos cells as proof of principle [Fig. 3].<sup>50</sup> They showed that 4000 Ramos cells can be seen by the naked eye,

and as low as 800 Ramos cells can be detected by a portable strip reader.

Apart from having SPR bands, gold nanoparticles also have the ability to quench the fluorescence of fluorophores through FRET or an electron-transfer mechanism.<sup>51</sup> By careful choice of gold nanoparticles, fluorophores, and ligands to assemble into a complex, target-induced fluorescence signal turn-on can be observed, thus leading to versatile and simple platforms for bioanalysis. Based on this strategy, considerable work has been done using aptamer-conjugated gold nanoparticles.<sup>52–54</sup> In one paper,<sup>52</sup> Huang *et al.* constructed platelet-derived growth factor (PDGF) binding aptamer-conjugated gold nanoparticles for sensitive detection of PDGF, using *N,N*-dimethyl-2,7-diazapyrenium dication (DMDAP) as a reporter. Mirkin's group found that polyvalent DNA functionalized gold nanoparticles, composed of a gold nanoparticle core (2–250 nm) and a dense shell of synthetic oligonucleotides (including aptamers), were capable of entering a variety of cell types with enhanced stability towards enzymatic digestion.<sup>55</sup> This finding rendered aptamer conjugated gold nanoparticles useful for a variety of challenging investigations, such as intracellular detection. By using nano-flares (gold-nanoparticle functionalized thiol-terminated aptamers hybridized with a short complementary Cy5-labeled reporter strand), specific intracellular ATP detection was achieved.<sup>54</sup> When the nano-flares met ATP inside living cells, the binding between ATP and the corresponding aptamers caused



**Fig. 3** (A) Schematic illustration of detecting Ramos cells on an aptamer-conjugated gold nanoparticle strip biosensor. Ramos cells are captured on the test zone through specific aptamer-cell interaction, while excess aptamer-conjugated gold nanoparticles are captured on the control zone through aptamer-DNA hybridization. (B) Typical photo images (top) and corresponding responses (bottom) of aptamer-conjugated gold nanoparticle strip biosensor with samples containing different amounts of Ramos cells (target cells) and CLL cells (control cells). From left to right: 0 Ramos cells;  $8 \times 10^4$  CCL cells;  $8 \times 10^4$  Ramos cells;  $8 \times 10^4$  CLL cells and  $8 \times 10^4$  Ramos cells. C = Capture zone; T = Test zone. The large signal in the capture zone is due to excess aptamer-conjugated Au nanoparticles. Adapted from ref. 50.

a conformational change in the aptamers, displacing the Cy5-labeled reporter strand to restore the fluorescence signal.

### Carbon nanotubes

Apart from these spherical nanomaterials, nanomaterials with other shapes also have great significance for bioanalysis. Among them, carbon nanotubes (CNTs), including single and multi-walled CNTs, have been extensively studied since their discovery in the 1990s.<sup>56</sup> They are mosaics of carbon atoms that form graphene sheets and curl into seamless tubules. This review focuses on single-walled carbon nanotubes (SWNTs). Recent research has found that SWNTs can act cooperatively as effective quenchers for a variety of fluorophores, even nearby quantum dots, through an energy or electron transfer process, resulting in low background and high signal-to-noise ratio for their use in biosensor development.<sup>57,58</sup> Researchers also found that SWNTs can interact with a variety of biomolecules covalently or non-covalently, such as proteins<sup>59,60</sup> and nucleic acids.<sup>61,62</sup>

With respect to nucleic acids, single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) interact with individual SWNTs by adopting different patterns.<sup>63,64</sup> Through  $\pi$ - $\pi$  stacking interactions between the nucleotide bases and the SWNT sidewalls, ssDNA, including aptamers, wrap around SWNTs with nearly complete DNA coverage to form stable complexes. On the other hand, dsDNA interacts relatively weakly with SWNTs, and the interaction is highly dependent on the surface charge of the SWNTs. That is, towards uncharged SWNTs, dsDNA interacts with either end because of the exposed hydrophobic bases. In contrast, for positively charged SWNTs, dsDNA is adsorbed with a roughly parallel configuration. As such, both modes are distinguished from the wrapping mode for ssDNA. In addition, target binding can also interrupt the interaction between aptamers and SWNTs.

A number of sensing events based on the assembly of fluorophore-labeled functional ssDNA and SWNTs have been reported.<sup>65,66</sup> In the absence of target (either DNA or other biomolecules), the strong interaction between ssDNA and SWNTs brings the fluorophore and SWNTs into close proximity, quenching the fluorescence signal of the fluorophore, which, in turn, causes low background. However, in the presence of target, specific binding to the functional ssDNA, either through DNA hybridization or formation of an ssDNA-target complex, leads to the dissociation of functional ssDNA from the SWNTs, resulting in restoration of the fluorescence signal. This principle

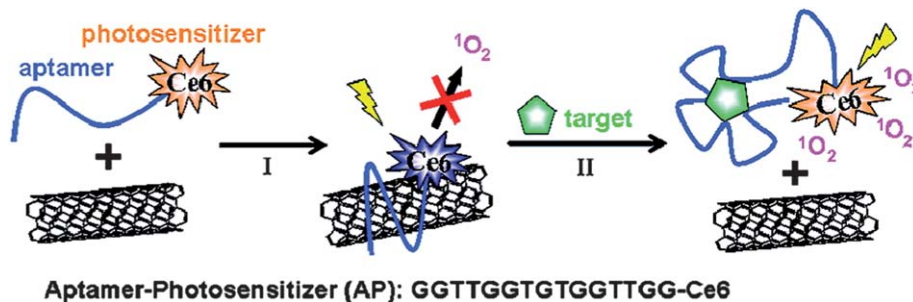
has opened new avenues in biosensor development. For example, Yang *et al.* harnessed this scheme for the detection of complementary DNA *via* a molecular beacon-SWNT complex.<sup>65</sup> With the help of SWNTs to reduce background and enhance the signal-to-background ratio, a detection limit of 4.0 nM, 8-fold lower than that of a normal molecular beacon, was achieved. The probe also had the ability to differentiate perfectly matched and single-base mismatched targets. In addition, compared to a normal molecular beacon, this molecular beacon-SWNT complex had much higher thermal stability. The method is cost effective, since no quencher is needed to prepare the molecular beacon, and further modification of the beacon is easy.

Zhu *et al.* engineered a novel photosensitizer-aptamer-SWNT complex to achieve singlet oxygen generation (SOG) regulation for photodynamic therapy (PDT) [Fig. 4].<sup>66</sup> In their demonstration, ATP and thrombin were used as small molecule and protein targets, respectively. In the absence of target, the photosensitizer Ce6 was brought into close proximity of the SWNT surface *via* normal aptamer wrapping, as described above. Since the fluorescence process and SOG share a similar photophysical mechanism, SOG is efficiently quenched at this stage. However, upon target binding, the conformation of the probe changes, thus disturbing the DNA interaction with the SWNT to remove the aptamer. This leads to the restoration of SOG upon illumination for PDT applications. In this manner, the highly specific regulation of SOG can be directly attributed to the high specificity of aptamers. Moreover, by simply changing the aptamer-target pair, this approach can be used as a general method for SOG regulation.

### Gold nanorods

Gold nanorods comprise another type of non-spherical nanomaterial widely used for biosensing. Gold nanorods are mainly synthesized by either the seed-mediated growth method<sup>67,68</sup> or the lithographic method,<sup>69,70</sup> although electrochemical<sup>71</sup> and catalytic<sup>72</sup> methods are also used. By conjugating with specific biomolecules, such as enzymes,<sup>73,74</sup> proteins,<sup>75</sup> or DNAs,<sup>76-78</sup> gold nanorods can be equipped with molecular recognition modules for biosensing. Changes in Raman spectra, bioconjugate stability<sup>79</sup> and zeta potential are commonly used to verify the successful attachment of biomolecules to gold nanorods.

Compared to gold nanoparticles, gold nanorods are especially intriguing in that they have two distinctive features. One is their relatively larger surface area.<sup>80</sup> The other is their dimensional



**Fig. 4** Schematic illustration of aptamer-photosensitizer-SWNT complex construction and the regulation of SOG upon target binding. Adapted from ref. 66.



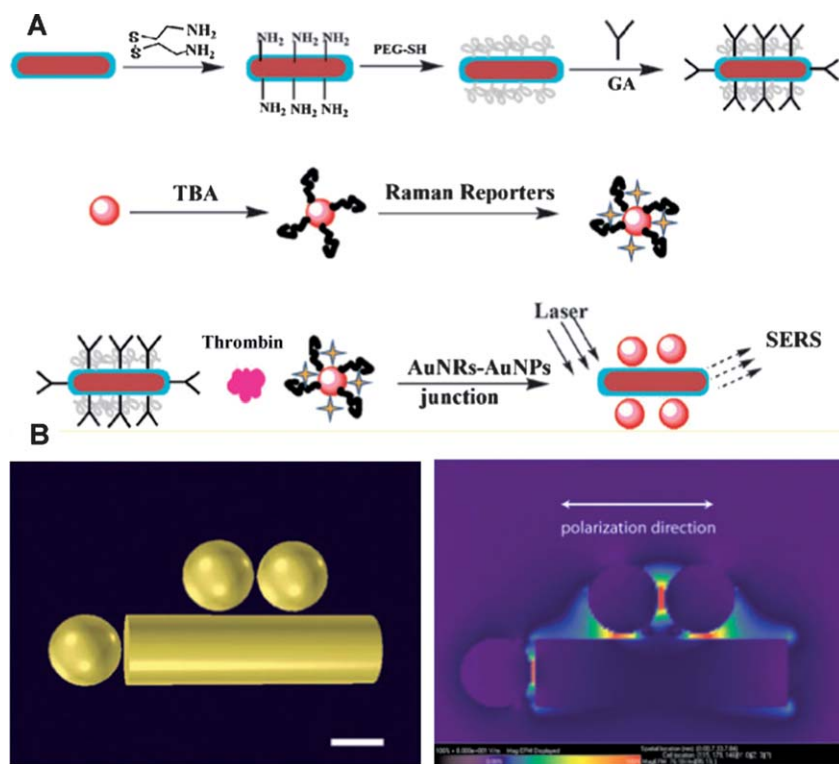
anisotropy, giving rise to a transverse as well as a longitudinal surface plasmon resonance (LSPR) band.<sup>81</sup> By reason of their large surface areas, it is easy to functionalize gold nanorods with multiple biomolecules. Huang *et al.* showed that up to 80 fluorophore-labeled aptamers can be conjugated onto a 12 nm \* 56 nm gold nanorod.<sup>82</sup> This resulted in a much stronger fluorescence signal and a much higher binding affinity toward target cells compared to an individual fluorophore-labeled aptamer. According to their results, this strategy is versatile, working not only for aptamers with strong binding capability (*e.g.*, Sgc8 for CEM cells), but also for aptamers with weak binding capability (*e.g.*, KK1H08 for K-562 cells). As a result of the enhanced fluorescence signal and improved binding affinity associated with multivalent binding, aptamer conjugated gold nanorods can be good candidates for more sensitive and specific bioanalyses. Regarding the LSPR band of gold nanorods, the wavelength can be tuned with ease from near infrared (NIR) to the infrared (IR) region by controlling the aspect ratio of the gold nanorods.<sup>83</sup> Recent theoretical<sup>84,85</sup> and experimental results<sup>86,87</sup> have shown that an enhanced Raman scattering signal can be produced from the large electromagnetic field generated by the SPR on metal nanostructures, making both gold nanoparticles and gold nanorods suitable candidates for surface enhanced Raman scattering (SERS) applications. This is attributed to the long-range electromagnetic field mechanism for SERS,<sup>88</sup> as well as the short-range chemical enhancement mechanism.<sup>89</sup>

As for the electromagnetic field mechanism, proximity to the metal surface and particle shape both play an important role in enhancing the Raman scattering signal.<sup>88</sup> An enhancement factor

of  $10^4$ – $10^5$  for adsorbed molecules on gold nanorods was observed, whereas no similar phenomenon was observed for gold nanoparticles.<sup>90</sup> Therefore, higher sensitivity can be achieved by using gold nanorods for biosensing based on SERS *in vitro* and *in vivo*. For example, Wang *et al.* proposed a novel SERS aptasensor based on gold nanorod-gold nanoparticle junctions to detect thrombin [Fig. 5].<sup>91</sup> In their work, gold nanorods were functionalized with thrombin-binding antibodies, while gold nanoparticles were conjugated with corresponding Raman reporter labeled aptamers. The interaction of thrombin with antibodies on gold nanorods and aptamers on gold nanoparticles brought the two nanostructures into close proximity, creating SERS hot spots for an enhanced Raman scattering signal. When there was no thrombin, no detectable signal was observed. By using this approach, 220 pM thrombin in Tris-HCl buffer and 887 pM thrombin in 5% human blood serum can be detected. By using different Raman reporters, this approach based on aptamer-protein recognition and hot spot formation can be used for multiplexed detection of rare proteins in blood samples.

### Aptamer-conjugated nanomaterials as delivery vehicles

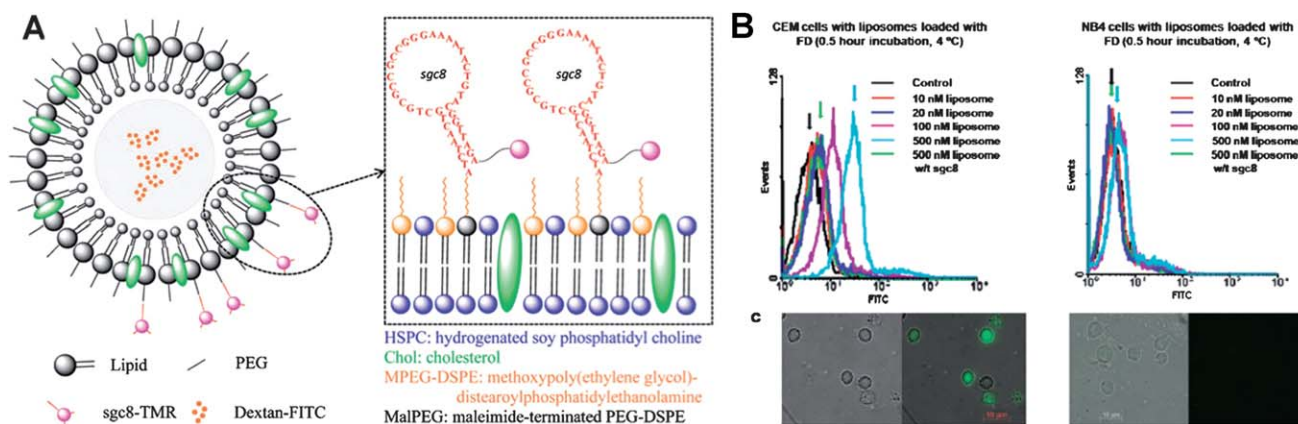
Apart from their use as sensing platforms for bioanalysis, nanomaterials can also be used for other biomedical purposes.<sup>92–95</sup> It is these advances in bionanotechnology that are fueling the shift from traditional symptomatologic medicine to future prospective medicine, which embodies predictive, preventive, personalized and participatory (P4 Medicine TM) medicine. Nanomaterials are



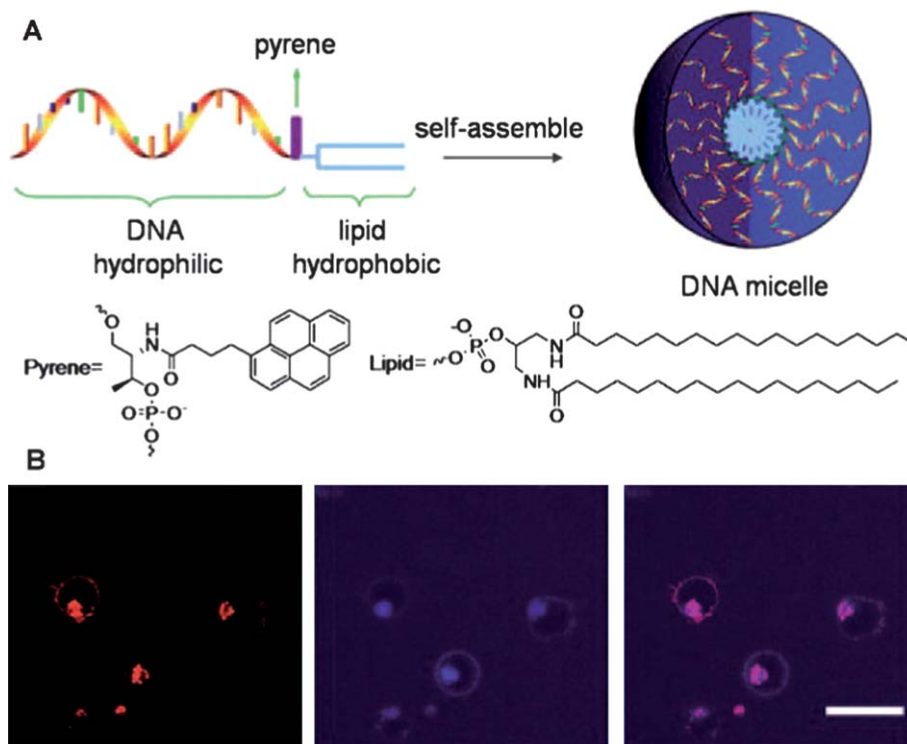
**Fig. 5** (A) Schematic illustration of gold nanorod-gold nanoparticle junction construction for thrombin detection, GA represents glutaraldehyde and TBA represents thrombin binding aptamer. (B) Finite difference time domain (FDTD) simulation and junction enhancement at the excitation laser wavelength of 632.8 nm. Adapted from ref. 91.

currently applied as delivery vehicles,<sup>96</sup> regulation moieties<sup>97</sup> and therapeutic agents.<sup>98</sup> When used as carriers, nanomaterials are bound with cargo molecules, such as drugs or functional proteins, either covalently or non-covalently. Covalent modifications of cargoes are generally achieved through standard gold-thiol chemistry,<sup>99</sup> peptide bond formation<sup>100</sup> or other methods.<sup>101</sup> Electrostatic adsorption<sup>102</sup> and hydrophobic interaction<sup>103</sup> schemes are commonly employed to introduce cargo molecules non-covalently. Regardless of the methods for cargo molecule introduction, the water-solubility and biocompatibility of nanomaterials themselves as delivery vehicles are crucial to their *in vivo* applications.

Compared with the solid nanomaterials described, soft nanomaterials (*e.g.*, liposomes and micelles) have high water-solubility, prolonged circulation time in the blood and enhanced accumulation at the tumor site.<sup>104</sup> In addition, most soft nanomaterials are prepared from the same or similar materials as cell membranes, and are therefore inherently biocompatible.<sup>105–107</sup> By introducing PEG molecules and other targeting ligands, non-specific binding between building blocks of soft nanomaterials and the cell membrane can be greatly reduced. Since most recognition events with target cells are achieved by the specific interaction between ligands and cell membrane receptors, soft



**Fig. 6** (A) Design scheme of multifunctional liposome nanostructure construction for targeted delivery. (B) Flow Cytometry histograms (top) and corresponding confocal microscopy images (bottom) of aptamer-conjugated liposome with target cells (left) and control cells (right). Adapted from ref. 110.



**Fig. 7** (A) Design scheme of DNA-micelle. (B) Localization and distribution of DNA-micelle inside CEM cells, from left to right: CEM cells treated with TAMRA-labeled DNA-micelles; CEM cells treated with Tf-Alexa 633; overlay of the first two channels. Adapted from ref. 114.



nanomaterials modified with aptamers generated from cell-based SELEX are superior in delivery.<sup>108,109</sup>

Using liposomes for drug delivery is already an established technology. Liposomes have been successfully used for the delivery of poorly soluble chemotherapeutics. However, targeted delivery with high specificity is still needed. To this end, Kang *et al.* linked aptamers to liposomes to build a multi-functional target specific delivery system [Fig. 6].<sup>110</sup> Briefly, hydrogenated soy phosphatidyl choline (HSPC), cholesterol (Chol), methoxy-poly-(ethylene glycol)-distearoyl-phosphatidyl-ethanolamine (MPEG-DSPE) and maleimide-terminated PEG-DSPE (Mal-PEG) with a ratio of 2 : 1 : 0.08 : 0.02 were mixed together to construct liposomes with MalPEG as the dock for Sgc8 aptamer covalent conjugation. To serve as a model drug as well as a reporter, FITC-Dextran was encapsulated into the Sgc8 aptamer-conjugated liposomes. Specific binding of the FITC-Dextran-loaded liposomes to their targeted cells was demonstrated by both flow cytometry histograms and confocal microscopy images.

Apart from liposomes, micelle aggregates composed of hydrophilic single-stranded DNA and hydrophobic polymers have also emerged as new delivery vehicles with good biocompatibility and high stability.<sup>111–113</sup> However, the coupling between the DNA and hydrophobic polymer is often inefficient, and the micelle sizes are not well controlled.<sup>111</sup> Liu *et al.* reported the easy synthesis of a well-defined DNA-diacyllipid micelle [Fig. 7].<sup>114</sup> The diacyllipid tail could be efficiently incorporated at the 5'-end of DNA through solid-phase oligonucleotide synthesis to construct the conjugate. In aqueous solution, the diacyllipid-DNA conjugates spontaneously self-assembled into mono-dispersed micelles with low critical micelle concentration (CMC) and high stability. When incubated with cells, these micelles dissociated, allowing the diacyllipid-DNA conjugates to enter living cells through endocytosis. They also found that the ability of micelles to permeate cells was size-dependent. Based on these features and properties, they envisioned that these DNA-micelles would be attractive for applications in bionanotechnology, cell biology and drug delivery. Based on their work, Wu *et al.* further explored the application of these superior DNA-micelles, especially as detection/delivery vehicles to cancer cells.<sup>115</sup> Instead of random ssDNA, the Td05 aptamer was introduced. In addition, they added a PEG linker between the lipid tail and the aptamer. It was found that the Td05 aptamer specifically binds to Ramos cells at 4 °C, but loses its binding affinity at 37 °C. However, the DNA-micelle composed of Td05-PEG-lipid conjugates showed strong binding to Ramos cells, even at 37 °C, with extremely low off rate (dissociation rate). Similar phenomena were also observed for other aptamer-micelles (*e.g.*, KK-micelles and KB-micelles). By doping Td05-micelle with CellTracker™ Green that only fluoresces inside the cell, a strong fluorescence inside Ramos cells was observed after only 2 h. In contrast, fluorescence with similar intensity for free CellTracker™ Green required 12 h. The aptamer-micelles were also flushed through a channel containing immobilized cancer cells, as a tumor site mimic, demonstrating that micelles have good recognition ability in whole blood samples. Taken together, these results showed that the DNA-micelle with enhanced binding affinity and unique internalization allows rapid molecular recognition of cancer cells and *in vivo* drug delivery.

## Outlook

Aside from silica and gold nanoparticles, carbon nanotubes and gold nanorods, many other nanomaterials, including silver nanowires and biodegradable nanomaterials, such as chitosan nanotubes<sup>116</sup> and chitosan dispersed carbon nanotubes,<sup>117</sup> will carry nanotechnology one step further in satisfying the demands of biomedical applications. Bioconjugates integrating these nanomaterials with aptamers will further mediate the development of bioanalysis and biotechnology. Particularly, in terms of multiplexing, the combination of four DNA bases can, for example, produce millions of DNA strands with different sequences to meet the needs of specific recognition towards the desired targets with high affinity. In addition, advances in DNA engineering are expected to make the design of aptamer-conjugated nanomaterials more widely available.

Other than their utilization as platforms for bioanalysis and carriers for drug delivery, nanomaterials will find more applications in the biomedical field. They have, for instance, been used as imaging agents for increased resolution and enhanced contrast.<sup>118–120</sup> Future efforts will focus on using aptamer-conjugated nanomaterials to achieve localized enrichment in order to further improve imaging resolution and contrast. Nanomaterials have also been applied to three-dimensional cell culture and tissue engineering.<sup>121–123</sup> For example, magnetic nanoparticles functionalized with appropriate ligands have been used to guide the direction of cell growth.<sup>124</sup> This line of research is still in its infancy and aptamer-conjugated nanomaterials will pave the way for this field based on the specific interaction between aptamers and cell membrane receptors.

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