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## Nucleic Acid Conjugated Nanomaterials for Enhanced Molecular Recognition

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### Abstract

Nucleic acids, whether designed or selected *in vitro*, play important roles in biosensing, medical diagnostics and therapy. Specifically, the conjugation of functional nucleic acid-based probe molecules and nanomaterials has resulted in an unprecedented improvement in the field of molecular recognition. With their unique physical and chemical properties, nanomaterials facilitate the sensing process and amplify the signal of recognition events. Thus, the coupling of nucleic acids with various nanomaterials opens up a promising future for molecular recognition. The literature offers a broad spectrum of recent advances in biosensing by employing different nano-platforms with designed nucleic acids, especially gold nanoparticles, carbon nanotubes, silica nanoparticles and quantum dots. The advantages of these novel combinations are discussed from the perspective of molecular recognition in chemistry, biology and medicine, along with the problems confronting future applications.

### Keywords

Molecular Recognition; DNA; Aptamers; Molecular Beacons; DNzyme; Gold Nanoparticles; Nanorod; Carbon Nanotubes; Silica Nanoparticles; Quantum Dots

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Molecular recognition is key in the design of sensors and switches, as well as the development of clinical diagnostic tools and therapeutic modalities. In early years, various organic molecules possessing unique properties drew the attention of investigators to achieve the recognition of different targets.<sup>1,2</sup> Particularly, since the discovery of the double helix structure of DNA,<sup>3</sup> the Watson-Crick type of hydrogen bonds, combined with electrostatic force,  $\pi$ -stacking and hydrophobic forces, have made it possible to design suitable probes for signaling biomolecular interaction<sup>4</sup> by the very nature of highly specific molecular recognition ability of nucleotide base pairs. In addition to Watson-Crick type of hydrogen bonds for base pairing in molecular recognition of nucleic acids, there are recently developed nucleic acid probes known as aptamers for the recognition of a wide array of targets ranging from small ions to proteins to cells and tissues. Aptamers are oligonucleic acids selected *in vitro* by a process termed systematic evolution of ligands by exponential enrichment (SELEX) for binding different targets.<sup>5, 6</sup> Thus, with the advent of aptamers, the previous application of nucleic acids for

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molecular recognition took a big step forward.<sup>7</sup> Compared with traditional chemical recognition mechanisms, such as host-guest chemistry, the interaction of nucleic acids is universal and easily modified. As a consequence, various biosensors and diagnostic methods have been developed based on the special recognition properties of nucleic acids.<sup>8</sup> Moreover, progress in the development of nanomaterials provides nucleic acids even more flexibility as molecular recognition tools.

Unlike other biomolecules, such as proteins, nucleic acid probes are more stable and flexible when they are used with modifications. That is, the ease of handling DNA base modification and DNA strands, when combined with the different modification strategies of nanomaterials, provides a vast platform upon which to build novel molecular recognition tools. This is illustrated by the broad application of DNA and nanomaterial conjugates in the fields of spectroscopy, electrochemistry, magnetics,<sup>9</sup> and others.<sup>10</sup> Such conjugates offer three important improvements in molecular recognition.

First, nanomaterials can facilitate signal transduction; that is, when suitable nanomaterials work as reporters, the signal of recognition events can be amplified by several orders of magnitude.<sup>11</sup> In this way, a number of reporters can be incorporated into a single nanoparticle, which can enhance signal transduction by thousands of times. Moreover, at the nanoscale, a single recognition event might break the balance between different nanoparticles. This event, in turn, could be accompanied by a change in the property of the whole assembly, resulting in a greatly amplified signal. This phenomenon can be demonstrated by the color change of gold nanoparticle solution caused by the unbalanced electrostatic interactions resulting from the introduction of a small interference. Second, nanomaterials may make recognition more effective. Nanomaterials can be modified according to the function of the designed DNA probes, particularly given their high ratio of surface area to volume. In addition, cooperative interaction, also known as synergism, plays a key role in molecular recognition. By definition, synergism is the combined effect of two or more like-acting components exceeding the sum of the effect of the components used alone.<sup>12</sup> In this way, cooperative interaction can ease the challenge of recognition towards targets that have more binding sites. Furthermore, nanomaterials can participate in the molecular recognition process by interacting with the DNA probe, which may also increase the DNA binding selectivity. Third, the unusual interactions between nanomaterials and living systems make the application of functional DNA more practical for molecular recognition in medical diagnostics. For example, with the help of nanomaterials, nucleic acids can escape nuclease digestion<sup>13</sup> and be transported across the cell membranes to recognize bioactive substances, thus allowing real-time monitoring of recognition events *in vivo*.<sup>14</sup>

As a consequence of these advantages, a combination of DNA molecular design and different nanomaterials will lead to enhanced, sometimes new, functions in molecular recognition. Today, there are many nanomaterials such as gold nanoparticles, carbon nanotubes, silica nanoparticles, quantum dots and magnetic nanoparticles which have been widely applied in the interdisciplinary fields of chemistry,<sup>15</sup> biology<sup>16</sup> and medicine.<sup>17</sup> It is from this perspective that we focus on how recent advances in nanomaterial conjugation improve the designed nucleic acid probes for molecular recognition.

## Gold Nanomaterials

Compared with either bulk metals or those of molecular compounds, metal nanomaterials display distinct physical properties<sup>18</sup> depending on material size, shape, surface function, and interval distance. Furthermore, the special interaction between mercapto-group and Au atom facilitates the modification of nanomaterials with oligonucleotides<sup>19</sup> and other compounds. By modifying these physical properties to meet the functional requirements of the DNA probe,

metal nanomaterials-conjugated oligonucleotides become ideal platforms for achieving efficient molecular recognition.

To report the DNA hybridization event, oligonucleotides were functionalized with gold nanoparticles. The mechanism of action is based on the distance-dependent surface plasmon absorption of gold nanoparticles.<sup>10, 20</sup> Thus upon the addition of target DNA, the probe DNA-modified dispersion of gold nanoparticles takes place, turning the solution from red to blue as a result of the hybridization of DNA functionalized gold nanoparticles by hundreds of target DNA. Due to the extremely high molar absorptivity of gold nanoparticles, 1000-times higher than that of organic dyes,<sup>21</sup> the DNA hybridization event is signaled and amplified.

In this method, it is the dispersing to aggregating movement, or *vice versa*, of the oligonucleotide-functionalized gold nanoparticles that causes the obvious and sensitive color change and, at the same time, facilitates signal amplification. Using this principle as a foundation, the application the DNA conjugates could be extended to recognize different molecules. Most notably, assembled gold nanoparticles in combination with aptamers make the aptamers be efficient for colorimetric recognition of targets based on the structural change of the aptamer upon target binding.<sup>22</sup> DNAzymes, which catalyze the hydrolysis of nucleic acids containing given sequences with cofactors, such as metal ions,<sup>23</sup> were also demonstrated to be an effective colorimetric probe by using gold nanoparticle. With the help of  $Pb^{2+}$ , the DNAzyme would cleave the substrate DNA, and gold nanoparticles facilitated the recognition event and signal transduction.<sup>24</sup>

If the target is much bigger in size than the aggregate of nanoparticles or contains more binding sites, the performance of molecular recognition could be improved by the action of synergy, which, as defined previously, is the combined effect of two or more like-acting components exceeding the sum of the effect of the components used alone.<sup>12</sup> This has been demonstrated by the gold nanoparticle-based colorimetric detection of platelet-derived growth factor (PDGF)<sup>25</sup> and cancer cells. Specifically, there are two sites for aptamer binding on PDGF, and they act like glue to crosslink the aptamer-labeled gold nanoparticles. Since this activity results in net aggregation of the nanoparticles and target, the accompanying absorbance change of the solution is more sensitive to the target, and PDGF at the nanomolar level can therefore be detected. By using cancer cell aptamers<sup>26</sup> and gold nanoparticles, direct colorimetric assay of cancer cells has also been achieved (Figure 1).<sup>27</sup> Since the volume of a given cancer cell is much larger than the aptamer-functionalized nanoparticles, many aptamers immobilized on gold nanoparticles can bind with one cell very fast; thereafter, the effect of synergy greatly enhances the recognition ability of the aptamers. Thus, target binding and gold nanoparticle assembly has been achieved simultaneously. As confirmed by TEM pictures, gold particles attached to and assembled on the surface of target cells caused the color to change.

The interactions between DNA strands and bare gold nanoparticles provide a convenient way for gold nanoparticles to not only signal and amplify the recognition event, but also participate in the recognition process. For example, gold nanoparticles show more strong affinity to single-stranded (ssDNA) than that of double-stranded DNA (dsDNA). The negatively charged backbones of adsorbed ssDNA provide more electrostatic repulsion to stabilize gold nanoparticles, while dsDNA has less ability to stabilize gold nanoparticles in high salt solution.<sup>28</sup> This different propensity of ssDNA and dsDNA to adsorb onto gold nanoparticles could enable the design of a label-free colorimetric approach for DNA hybridization assay.<sup>29</sup> Specifically, since the electrostatic balance is easily broken by the small disturbance caused by the hybridization of DNA, recognition events can be amplified by the aggregation of the whole nanoparticles.<sup>30</sup> Metal ions,<sup>31–33</sup> protein,<sup>34</sup> and other molecules<sup>35, 36</sup> can also be detected by the noncovalent assembly of gold nanoparticles and functional oligonucleotides. .

Gold nanoparticles also play an important role in overcoming difficulties encountered in using nucleic acids-based fluorescent probes. One challenge in designing DNA fluorescent probes, such as molecular beacon (MB) and fluorescence signaling aptamer, is the several variables that can compromise the increment of signal change upon interacting with the targets. These primarily include (1) selection of dye-quencher properties, (2) means of attachment of dye-quencher groups, (3) unidentifiable target binding sites, and (4) unforeseen conformational changes. As a consequence of specific electronic properties, gold nanoparticles are good quencher of a fluorophore.<sup>37</sup> By applying gold nanoparticles as a substitute for organic quenchers, using either covalent or noncovalent modification with the DNA, the quenching efficiency could be improved greatly, providing a more efficient method for fluorescent detection of DNA,<sup>38–40</sup> protein<sup>41</sup> or metal ion.<sup>42</sup>

As anisotropic nanoparticles with different aspect ratios, gold nanorods can be easily synthesized and immobilized with huge numbers of functional oligonucleotides. The advantage of coupling gold nanorods with a DNA molecular probe design is the large absorption cross section at the near-infrared (NIR) range, which provides for the development of a novel photothermal transformer for therapy.<sup>43</sup> Moreover, compared to individual oligonucleotide probes, functionalizing one nanorod with several oligonucleotides significantly improves the ability to signal the binding event. This improved performance was confirmed by conjugating an aptamer, with only weak binding affinity to cancer cells, and a gold nanorod.<sup>44</sup> Flow cytometry analysis showed 300-fold fluorescence intensity enhancement was achieved by using the nanorod-conjugated DNA probe in comparison with single aptamer molecule. The evolved recognition ability of the aptamer-conjugated nanorod was further demonstrated by its photothermal effect (Figure 2).<sup>45</sup> With excellent absorption in the NIR range, which overlaps the spectrum of minimum extinction of animal tissues, the aptamer-functionalized Au-Ag nanorod conjugate selectively bound to the target cell with enhanced affinity. After exposure to NIR light irradiation, the nanorod-bound cancer cells were killed by the localized heat produced by photothermal conversion, while the control cells remained live.

In summary, by their unique structural and optical features, DNA- functionalized gold nanomaterials make a highly useful platform for molecular recognition with promising applications in optical detection,<sup>46</sup> plasmonic imaging,<sup>47</sup> and surface-enhanced resonant Raman analysis.<sup>48, 49</sup>

## Carbon Nanotubes

Carbon nanotubes (CNTs),<sup>50</sup> which can be divided into single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs), are another important type of nanomaterials with which to improve DNA molecular recognition, in this case, by their perfect cylindrical structure and unique mechanical, electrical, and optical characteristics.<sup>51–53</sup>

Various complexes, including DNA strands, can be adsorbed noncovalently onto the sidewalls of CNTs by means of  $\pi$ - $\pi$  stacking interaction between nucleotide bases and the sidewalls of SWNTs,<sup>54–56</sup> which facilitates the application of CNTs-conjugated oligonucleotides for molecular recognition. Because the native fluorescence of the nanotube<sup>57</sup> is influenced by adsorbed DNA, SWNTs was employed to signal the DNA hybridization in aqueous solution,<sup>58</sup> even though the DNA hybridizing process was slow. Moreover, with the unique optical property of SWNTs, ordinary environmental interference against selective recognition was weakened, making it possible to apply this technique for DNA conformational polymorphism detection, even in whole blood, tissue, and inside living cells.<sup>59</sup>

CNTs are also good candidates to improve the recognition performance of fluorescent DNA probes. Photophysical studies have demonstrated that SWNTs can act collectively as quenchers for fluorophores or fluorophore- labeled ssDNA<sup>14, 60, 61</sup> by SWNTs, through energy-transfer

and electron-transfer processes.<sup>51</sup> With their rigid structure and hybridized bases, dsDNA, however, shows less adsorption to SWNTs than does ssDNA.<sup>55</sup> When combined with the quenching effect of CNTs, this difference could be used to improve the molecular recognition performance for DNA and protein. The key features of this design are as follows (Figure 3).<sup>62, 63</sup> First, as noted above, ssDNA molecules wrap around individual SWNTs by means of  $\pi$ -stacking interactions between the nucleotide bases and the SWNT sidewalls. Next, because the SWNTs act as both a “nanoscaffold” for the ssDNA and a “nanoquencher” of the fluorophore, only one end of the ssDNA must be labeled with a fluorophore. Under these conditions, the ssDNA molecules self-organize on the surface of the carbon nanotubes, completely quenching the fluorophore. Finally, in the presence of a target, competitive binding of the target and the carbon nanotubes with the ssDNA suppresses the fluorescence quenching, allowing fluorescence-signal enhancement that is large relative to that without a target. This combination of properties results in fluorescence enhancement that is sensitive and specific to the perfectly complementary ssDNA. Furthermore, this design, which is based on a simple, cost-effective synthesis, was shown to have a large signal-to-background ratio, high thermostability, and exceptional DNA-binding selectivity. Therefore, from the standpoints of design and engineering, production, and overall function, self-assembled ssDNA-SWNT complexes can easily replace conventional MBs, which provided new opportunities in the design of nanodevices for molecular recognition. For instance, the recognition event can also be reported by light scattering signals,<sup>64</sup> moreover the performance of this method could be improved by employing a non-labeled DNA fluorescent dye, such as ethidium bromide (EB).<sup>65</sup> As a planar molecule, EB can adsorb on the sidewall of SWNTs, reducing the background fluorescence as much as the quenching effect of SWNTs. The adsorbed EB preferred to intercalate the hybridized bases, and the fluorescence recovered after hybridization, thus greatly enhancing S/B.

The novel interaction between CNTs and DNA increases the application of the conjugates for molecular recognition in other areas. For instance, the aptamer/SWNTs conjugate has been used to regulate the generation of singlet oxygen.<sup>66</sup> In this case, the excited state of a photosensitizer can be quenched by SWNTs, and such quenching effect then inhibits the generation of singlet oxygen. However, upon binding with target, the photosensitizer-labeled aptamers are released from the sidewall of SWNTs, generating a considerable amount of singlet oxygen. The target protein-directed singlet oxygen generation is thereby accomplished, which demonstrates how DNA-functionalized SWNTs, with their excellent photothermal properties, have great potential for diagnostics and therapy.<sup>14</sup>

The possibility of using the CNTs-conjugated nucleic acids for diagnostics in cells was also illustrated by the satisfactory performance of a DNA probe-conjugated CNT for the recognition of specific cellular RNA.<sup>13</sup> Since DNA is easily digested by cellular enzymes,<sup>67</sup> a fluorescent DNA probe for the detection of manganese superoxide dismutase (MnSOD) mRNA was used to complex with SWNTs. The result of PAGE indicated that SWNTs protected ssDNA from cleaving, even after incubating 60 minutes with DNase I,<sup>13</sup> which can unselectively cleave ssDNA or dsDNA. The capability of the complex probe was further demonstrated in a cellular environment compared with free DNA probe.

## Silica Nanoparticles

With well-defined morphology and porosity, silica colloid was first prepared and characterized by Stöber et al.<sup>68</sup> As the native feature of silica, various hybrid silica nanoparticles, such as dye-doped fluorescent silica nanoparticles<sup>69</sup> and magnetic silica nanoparticles,<sup>70</sup> with distinct properties are prepared, making silica nanoparticles good candidates for constructing hybrid materials which can load and transport different agents for applications in different fields.<sup>71</sup>



The combination of hybrid silica nanoparticles with functional oligonucleotides offers great improvements in molecular recognition, particularly for sensitive reporting. Because large numbers of luminescent dyes can be encapsulated inside, the as-prepared dye-doped silica nanomaterials have promising advantages for amplifying the recognition signal over their counterparts with high intensity and excellent optical stability.<sup>72</sup> More important, unlike quantum dots and metal nanoparticles, the luminescent nanoparticles are more hydrophilic, biocompatible, and relatively more stable under different conditions, all of which make them excellent candidates for applications with functional nucleic acids *in vivo*.<sup>73</sup>

As a consequence of their signal amplification, dye-doped silica nanoparticles modified with oligonucleotides can be applied for ultrasensitive DNA detection (Figure 4).<sup>74</sup> Traditionally, one DNA probe could be labeled with only one or a few fluorophores, resulting in limited fluorescent signal. By contrast, since one silica nanoparticle can trap hundreds of fluorophores, an intense fluorescent signal, which is approximately  $10^4$  times higher compared with that of the single fluorophore-labeled DNA probe, from the trapped fluorophores can be obtained upon target recognition.<sup>75</sup> In addition, the silica shells can protect doped dyes from photodamage by minimizing oxygen through the outer environment. Based on the designed sandwich assay, target DNA in the subfemtomolar range can be detected. The signal amplification effect of the hybrid nanoparticle was also proved in genechips (Figure 5).<sup>76</sup> The DNA immobilized doped silica nanoparticle was used as a staining probe, which greatly enhanced detection sensitivity and photostability when compared to the traditional fluorescent protein streptavidin-phycoerythrin.

Moreover, fluorophores with different emission properties can be trapped in the matrix of silica nanoparticles for multiplex signaling.<sup>77</sup> By varying the ratio of doped fluorophores, nanoparticles exhibit multiple colors with one single wavelength excitation based on fluorescence resonance energy transfer,<sup>78</sup> which could be applied for multiple cancer cell recognition. For instance, aptamers for different cancer cells were immobilized onto the surface of silica-coated magnetic nanoparticles. Meanwhile, different dye-doped silica nanoparticles were also labeled with aptamers to report the binding of particular types of cancer cells. After magnetic washes, the collected samples were imaged, and the amounts of three different types of cancer cells were determined simultaneously (Figure 6).<sup>79</sup>

Another important hybrid material conjugated with DNA used for molecular recognition is magnetic silica nanoparticles. Magnetic nanomaterials are excellent tools as contrast agents for magnetic resonance imaging (MRI) and for use in separation and drug delivery.<sup>80</sup> Silica-coated magnetic nanoparticles have been synthesized in water/oil microemulsion.<sup>70</sup> Disulfide bond and biotin-avidin linkage<sup>81</sup> were successfully used for labeling nucleic acids with silica magnetic nanoparticles to concentrate, separate and diagnose different targets. MBs could be combined with magnetic silica nanoparticles to extend their application in separation. MBs-labeled magnetic nanoparticles are efficient tools for separation and collection of DNA trace amounts from a complex mixture. At the same time, the collection process can be monitored in real time from the fluorescence enhancement of the MBs.

The stable and facile synthesis of aptamers makes this versatile DNA-functionalized silica nanoparticle-based method ideal for biological applications and diagnostics. Particularly, magnetic separation, coupled with the highly selective properties of aptamers, is efficient for target collection and enrichment in complex clinical samples. In addition to efficient separation, dye-doped silica nanoparticles can be used to monitor the recognition event and provide signal enhancement. Recently, this novel magnetic separation method was applied to rapid cancer cell collection and detection.<sup>82</sup> To achieve both separation and monitoring, carboxyl-functionalized dye-doped nanoparticles were covalently linked with an aptamer for the cancer cells, while silica-coated magnetic nanoparticles were immobilized with aptamers via biotin-

avidin interaction. After three magnetic extractions, 40% of spiked target cancer cells were counted, consistent with extraction efficiency values of the immunomagnetic method.

## Quantum Dots

Quantum dots (QDs) or inorganic semiconductor nanocrystals are another kind of important nanomaterials, which have involved deeply in biological analysis<sup>83</sup> and imaging owing to their distinct photophysical properties, such as size-dependent stable luminescence properties, high quantum yields, broad absorbance bands but narrow emission spectrums.<sup>84</sup> In 1998, two pioneer works about water-soluble QDs inspired the succedent applications of quantum dots for molecular recognitions.<sup>85, 86</sup> QDs integrated with functional nucleic results in obvious evolution of molecular recognition, especially in the fields of multiplexed targets detection and single molecule/particle analysis.

Hydroxylated QDs were first immobilized with probe DNA to fluorescent monitor the in situ hybridization event with Y chromosome in human sperm cells.<sup>87</sup> The organic fluorophores of MBs can be replaced by QDs, to achieve better photostability, which could be used for longer time imaging.<sup>88</sup> Preliminary siRNA screening was also demonstrated by using the DNA conjugated QDs.<sup>89</sup> The specific but stable optical features make QDs based DNA probes superior in molecular recognition. Incorporate with aptamers, the functional QDs were successfully applied to fluorescent detect ATP,<sup>90</sup> thrombin,<sup>91, 92</sup> PDGF,<sup>93</sup> and cancer cells.<sup>94–96</sup>

Compared with the stable but complicated covalent procedures for immobilizing of functional nucleic acids with of QDs, electrostatic self-assembling of DNA probes and QDs provides more versatile scaffold to establish molecular recognition. The prepared negatively charged QDs could form compact complex with probe DNA in the presence of cationic polymer, exhibiting the strong FRET between QDs and the dyes on the DNA probes. After the hybridization, as the rigid dsDNA, the distance of energy transfer changed which reduced the efficiency of FRET. The hybridization event was detected by the changes of FRET between QDs and dye labeled DNA.<sup>97</sup> Modifying the surface of QDs with positively charged groups simplified the process of assemble, and the cationic polymer linker was avoided.<sup>98</sup>

Due to the broad absorption bands, QDs with different emissions can be excited simultaneously, which provides chances to recognize and monitor different targets at the same time after excitation by one source.<sup>99</sup> ZnS-capped CdSe QDs of different sizes were successfully incorporated into polymer beads with different ratios. Conjugated with different DNA probes, multiplexed DNA recognitions were demonstrated by using triple-color encoded QDs-tagged beads.<sup>100</sup> When QDs with different emissions were combined with aptamers, different target molecules could be recognized at the same time. Simultaneously cocaine and adenosine detection has realized by using the aptamers conjugated QDs assemblies.<sup>101</sup> The electrochemical multiplex targets analysis was also achieved by the DNA functionalized QDs.<sup>102, 103</sup>

With high quantum yields and anti-photobleaching properties, conjugations of QDs and functional nucleic acids are wonderful candidates to investigate the molecular recognition events in single molecule/particle level, which supply more real time information and improved sensitivities.<sup>104</sup> In a designed sandwiched assay, QDs not only acted as donors of FRET pairs, but also provided a nanoplatform to confine numbers of captured targets and amplify the signals, 100-fold greater than the conventional assays detected by confocal fluorescence spectroscopy. Similar aptamer coupled single-particle based assays were used to detect cocaine,<sup>105</sup> and to study the interaction between RNA and protein to screen inhibitors.<sup>106</sup>

## Conclusion and Perspective

The combination of nanomaterials and functional nucleic acids is universal and shows excellent performance for molecular recognition. The powerful functional nucleic acids act as the recognition part,<sup>107</sup> and different nanomaterials supply a powerful nano-platform to assist oligonucleotides to improve their ability in recognizing target molecules from complex samples with high sensitivity and selectivity.

Most of the presented advances are *in vitro* studies, succeeding in solving some problems of molecular recognition, including, for example, ultrasensitive detection, signal amplification, and enhanced recognition. However, existing shortcomings remain to be addressed. For instance, it has been found that aggregation of nanomaterials in a complex environment, especially living systems, greatly depressed the effectiveness and the nonspecific adsorption of some molecules, such as proteins, consequently producing a disturbance that leads to false results. Moreover, the potential toxicity of some nanomaterials to the human body is still not very clear.<sup>108</sup>

To overcome these challenges, surface modifications and improved hybrid nanomaterials are considered. After immobilization with different functional and biocompatible compounds, nanomaterials would produce fewer agglomerations and less injury towards cells, while still showing more effectiveness for research *in vivo*.<sup>109, 110</sup> Meanwhile, the ordinary procedure for assembling nanomaterials and oligonucleotides should help to facilitate the recognition of target molecules. DNA-templated or as-prepared hybrid nanomaterials might also be a solution.<sup>111, 112</sup>

Some novel detection techniques also emerge and show advantages when fabricated with the functional hybrid DNA nanomaterials in the field of molecular recognition. For example, the semiconductor CNTs<sup>113</sup> and silica nanowires<sup>114</sup> based field-effect transistors (FETs) are label-free, reusable and high sensitive, which get good performances in the detection of DNA,<sup>115, 116</sup> proteins<sup>117, 118</sup> and *Escherichia coli*<sup>119</sup> by modification with different DNA or aptamers. This kind of newly developed methods greatly supports the wider application of the functional nucleic acid conjugated nanomaterials.

With further improvements, these hybrid materials will have more significant impact in bioanalysis and exhibit attractive potential for further applications, such as diagnostics, drug screening, molecular therapy, and efficient drug delivery.

### Vocabulary

#### Molecular beacons

artificial single stranded oligonucleotides designed with stem-loop structures, which comprise a fluorophore and a quencher moiety at two opposite ends. Without target molecules, the base pairs of stem portion hybridize to hold the fluorophore and quencher close and the fluorescence is quenched. In the presence of target, the loop DNA region can bind to it and cause the stem-loop structure open, which would spatially separate the fluorophore from the quencher and the fluorescence increase.

#### Aptamer

*in vitro* selected short single stranded DNA or RNA with high binding affinity and specificity to various target molecules by folding into defined tertiary structures. Aptamers for different targets can be produced from random-sequence DNA or RNA libraries by a process called SELEX after a few rounds of affinity selection and amplification.



**DNAzyme**

catalytic DNA molecule, also called DNA enzyme or deoxyribozymes, which is selected in vitro from random sequence DNA pools. With the help of particular cofactors, such as metal ions and hemin, this DNA-based biocatalyst facilitates the chemical reaction of the substrates.

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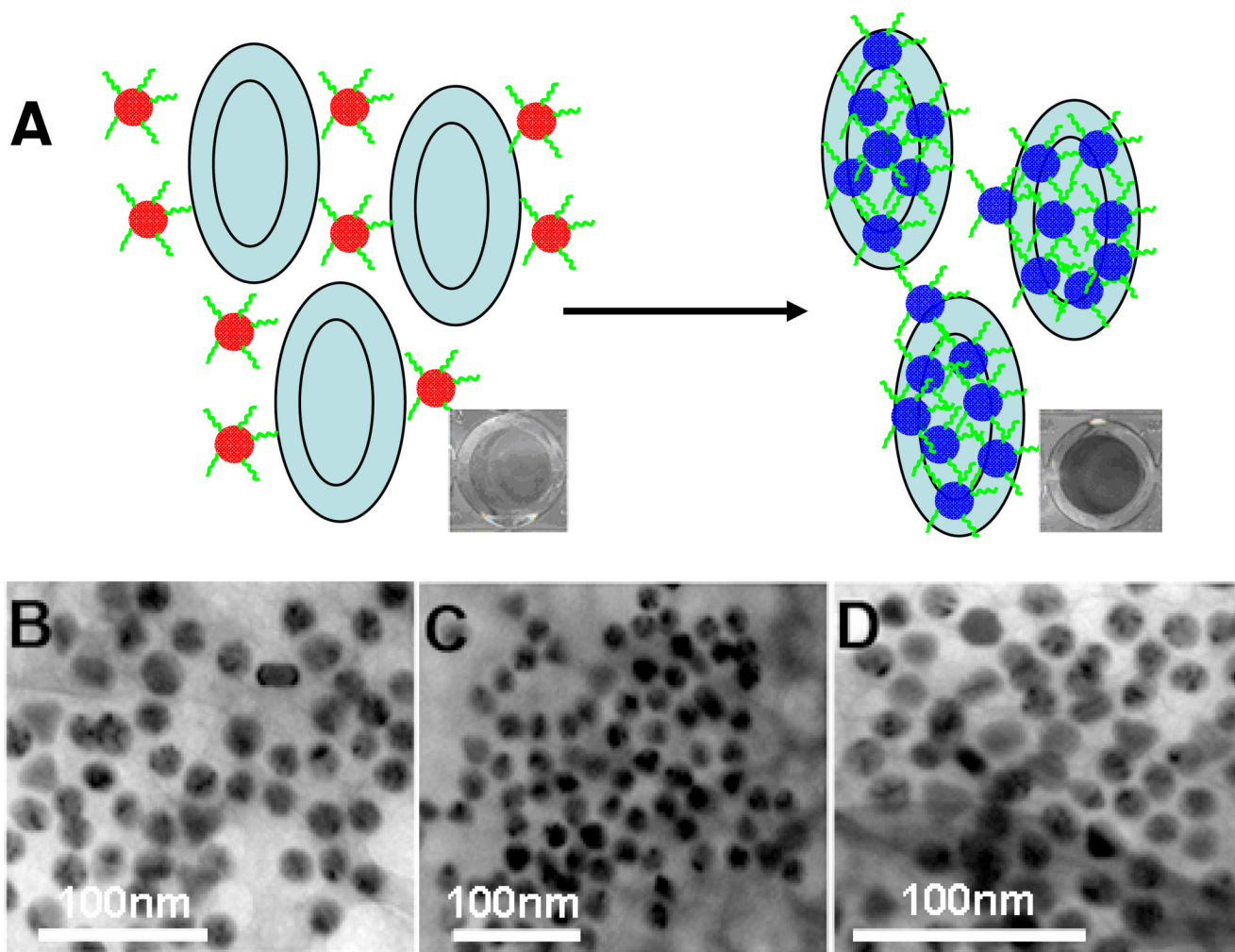
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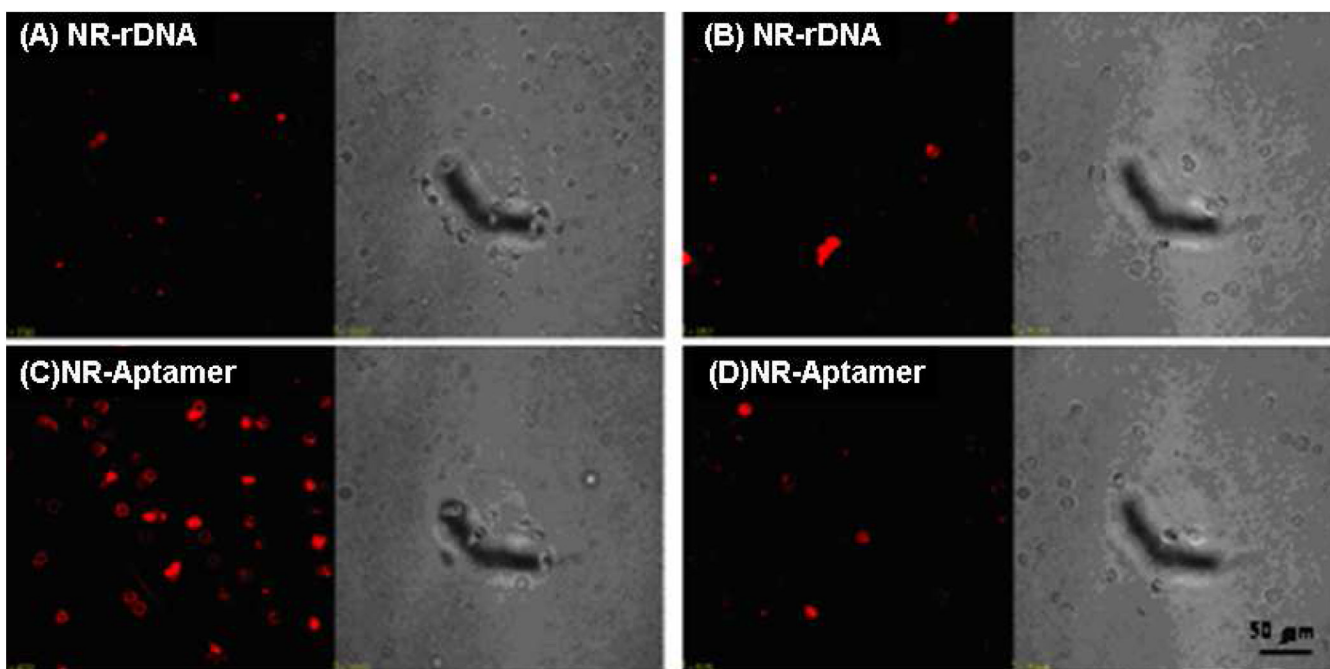
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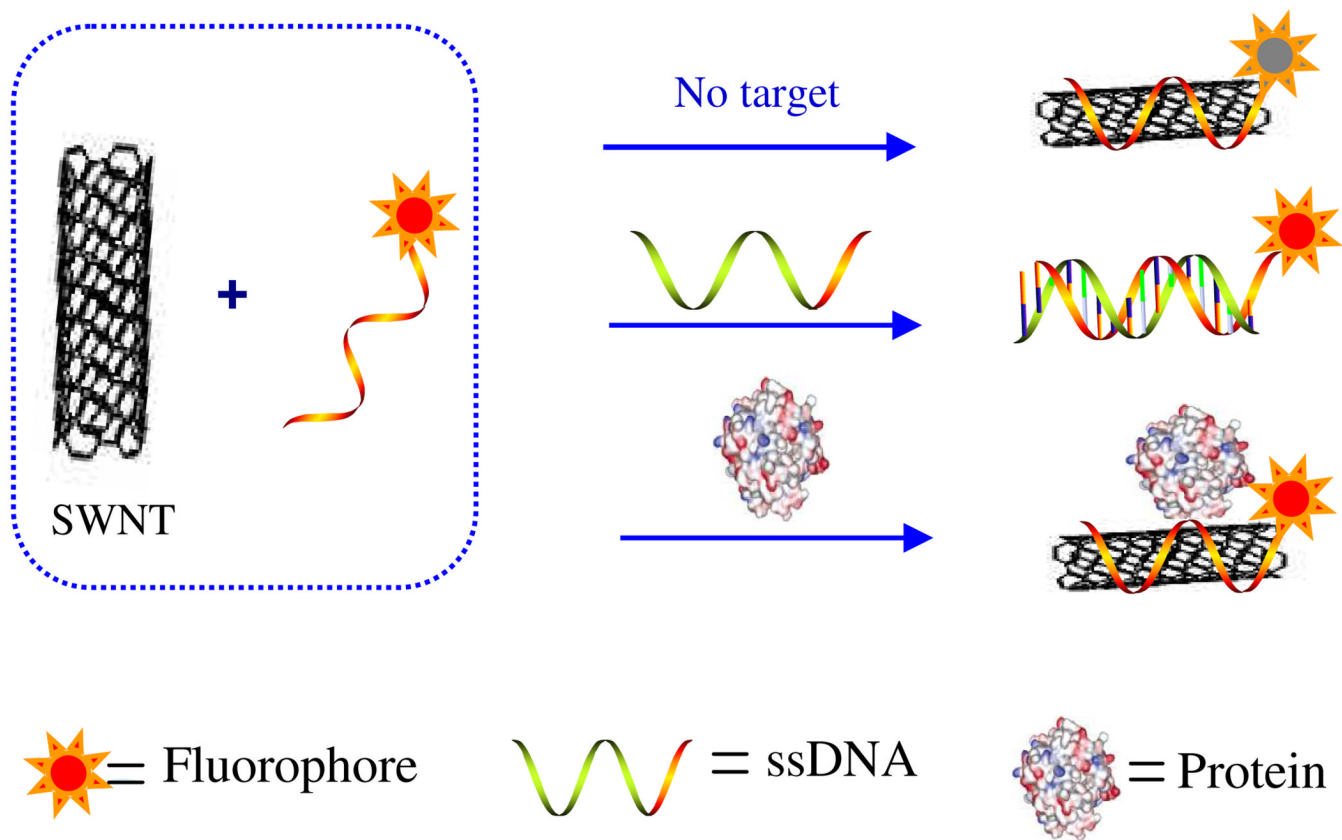
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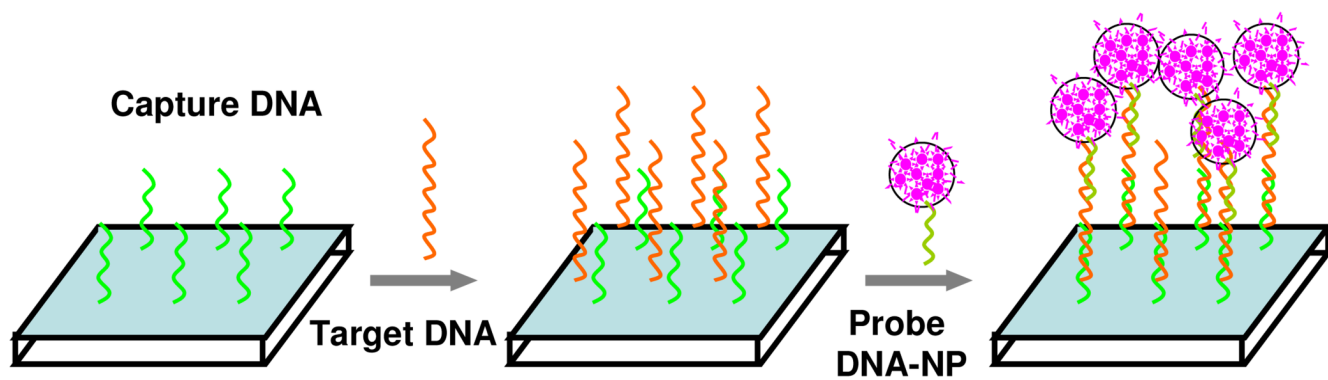
**Figure 1.** (A) Schematic of the colorimetric assay for cancer cells recognition based on the aptamer functionalized gold nanoparticle. (B–D) TEM images show the binding and assembling of aptamer-functionalized gold nanoparticles on different regions of the target cancer cell surface. Adapted from ref <sup>27</sup>.



**Figure 2.** Binding assay of nanorod-aptamer conjugates (NR-Aptamer) towards target cells (C) and control cells (D). The confocal images of target cells (A) and control cells (B) stained by random DNA conjugated nanorod (NR-rDNA) show less fluorescence. The scale bars are 50 $\mu$ m. Fluorescence images (left) and optical images (right). Adapted from ref <sup>45</sup>.

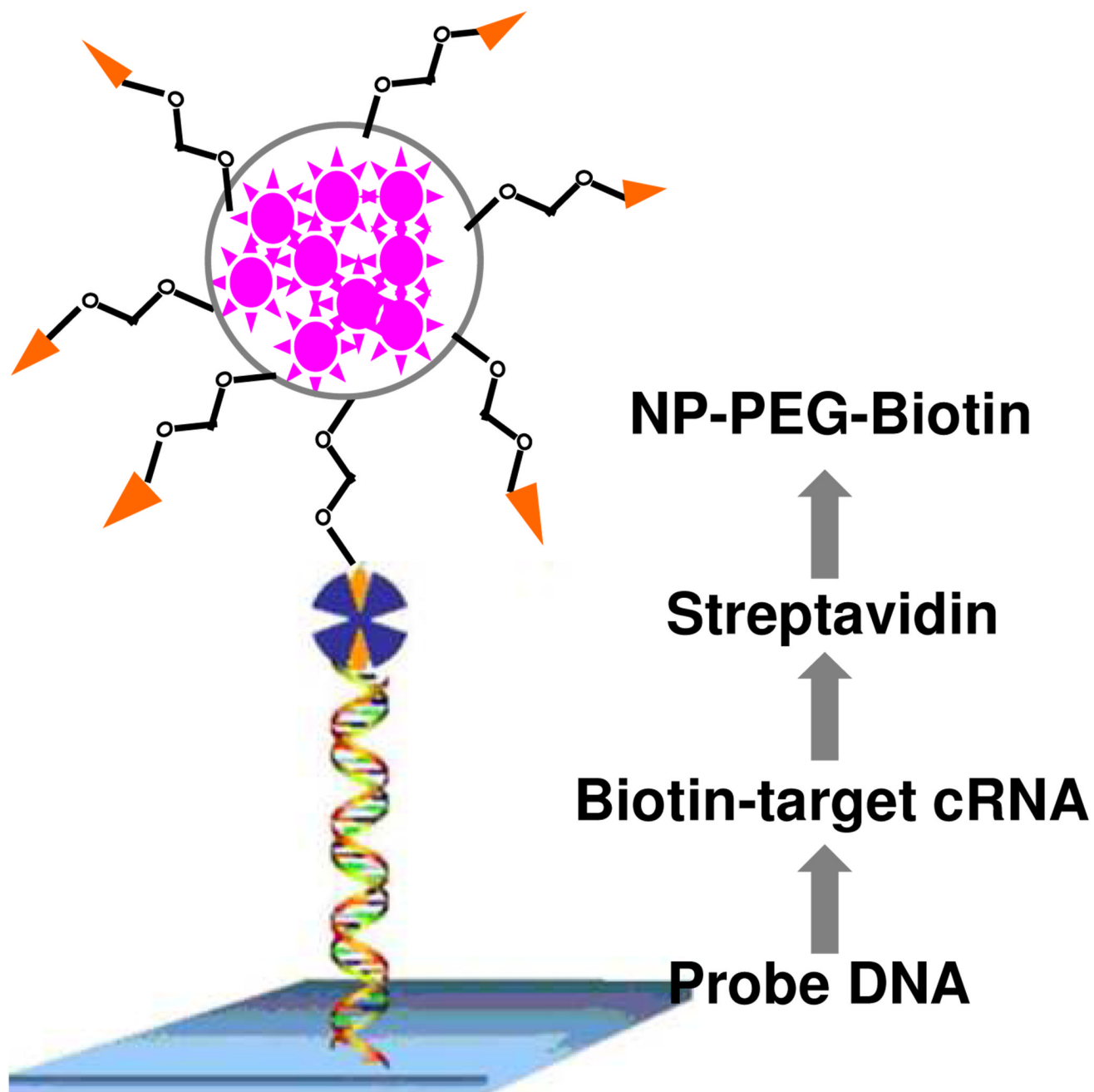


**Figure 3.** Scheme for signaling biomolecular interactions by the assembly of SWNTs and fluorophore-labeled ssDNA. Adapted from ref <sup>63</sup>

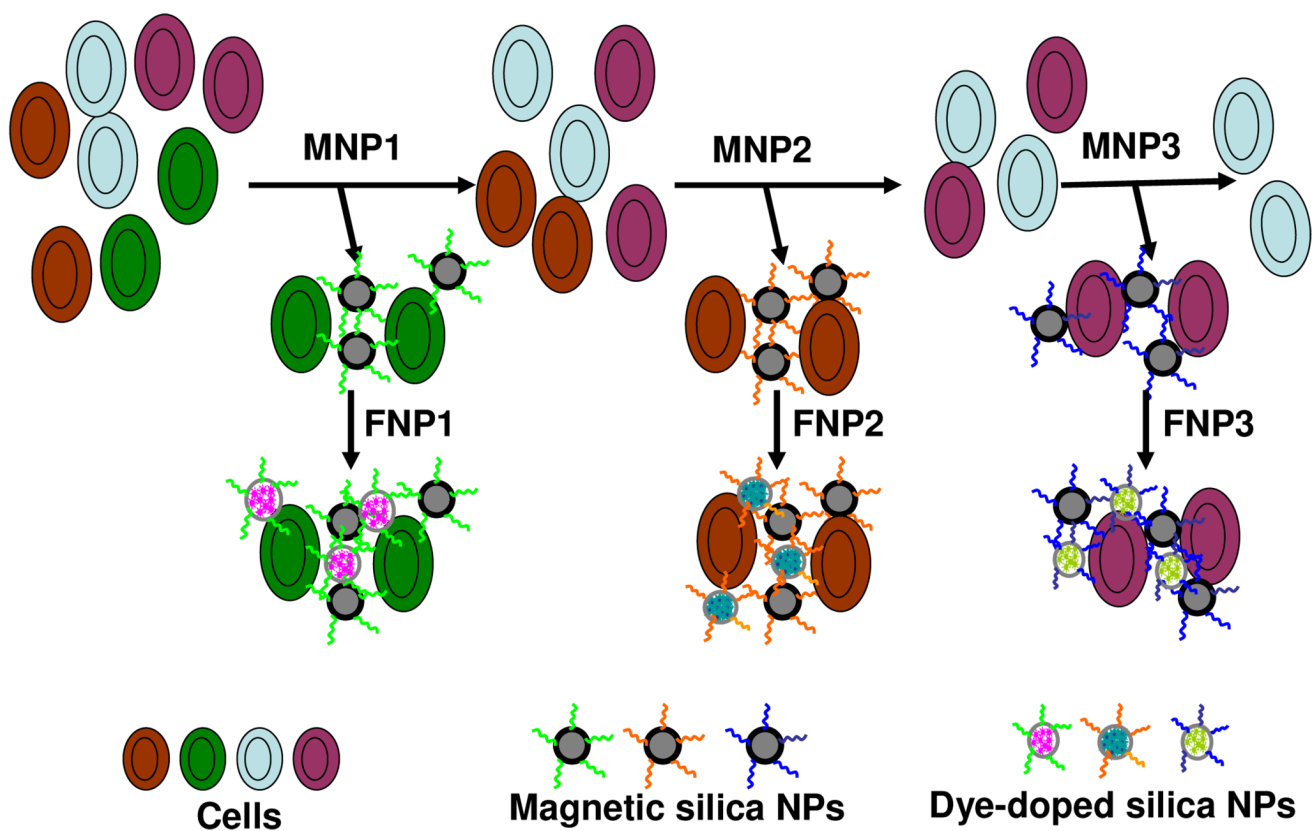


**Figure 4.** Schematic of a sandwich DNA assay based on dye-doped silica nanoparticles. Adapted from ref<sup>74</sup>





**Figure 5.** Strategy of dye-doped silica nanoparticle based labeling for genechip technology. Adapted from ref <sup>76</sup>



**Figure 6.** Schematic representation of the multiple extraction procedure with the magnetic silica nanoparticles being added and extracted stepwise and the corresponding dye-doped silica nanoparticles being added post-magnetic extraction of cell samples. Adapted from ref <sup>79</sup>.