

# Nanoscale

rsc.li/nanoscale



ISSN 2040-3372



ROYAL SOCIETY  
OF CHEMISTRY

Celebrating  
IYPT 2019

REVIEW ARTICLE

Nguyen Thi Kim Thanh, Xiaodi Su *et al.*  
Sensing of circulating cancer biomarkers with metal  
nanoparticles



NCNST



Cite this: *Nanoscale*, 2019, **11**, 22152

## Sensing of circulating cancer biomarkers with metal nanoparticles

Roger M. Pallares,  <sup>a,b,c</sup> Nguyen Thi Kim Thanh  <sup>\*a,b</sup> and Xiaodi Su <sup>\*c,d,e</sup>

The analysis of circulating cancer biomarkers, including cell-free and circulating tumor DNA, circulating tumor cells, microRNA and exosomes, holds promise in revolutionizing cancer diagnosis and prognosis using body fluid analysis, also known as liquid biopsy. To enable clinical application of these biomarkers, new analytical tools capable of detecting them in very low concentrations in complex sample matrixes are needed. Metal nanoparticles have emerged as extraordinary analytical scaffolds because of their unique optoelectronic properties and ease of functionalization. Hence, multiple analytical techniques have been developed based on these nanoparticles and their plasmonic properties. The aim of this review is to summarize and discuss the present development on the use of metal nanoparticles for the analysis of circulating cancer biomarkers. We examine how metal nanoparticles can be used as (1) analytical transducers in various sensing principles, such as aggregation induced colorimetric assays, plasmon resonance energy transfer, surface enhanced Raman spectroscopy, and refractive index sensing, and (2) signal amplification elements in surface plasmon resonance spectroscopy and electrochemical detection. We critically discuss the clinical relevance of each category of circulating biomarkers, followed by a thorough analysis of how these nanoparticle-based designs have overcome some of the main challenges that gold standard analytical techniques currently face, and what new directions the field may take in the future.

Received 9th April 2019,  
Accepted 24th August 2019

DOI: 10.1039/c9nr03040a

rscl.li/nanoscale

### 1. Introduction

Tissue biopsy is a gold standard technique for the investigation and diagnosis of cancer. Nevertheless, the removal of patient tissue presents important limitations in terms of sample acquisition and information obtained. As an example, one of the main challenges in tissue biopsy is cancer heterogeneity,<sup>1</sup> which can exist within the same tumor (intra-tumoral heterogeneity) and between metastases in the same patient (inter-metastatic heterogeneity).<sup>2</sup> Therefore, the extraction of tissue from a specific solitary tumor may not provide the whole information of the patient condition. However, multiple tissue extractions are not recommended to overcome cancer heterogeneity because every extraction increases the risk of spreading the disease to other parts of the body,<sup>3</sup> and tissue

biopsies present clinical complications (*e.g.* 17.1% of thoracic biopsies have been reported to result in adverse events<sup>4</sup>). Furthermore, the location of some tumors makes the sample extraction very challenging or impossible.<sup>5</sup>

Liquid biopsy, as an alternative to tissue/solid biopsy, is less invasive and more robust against cancer heterogeneity. The analysis and quantification of cancer-related biomarkers from various body fluids, including blood,<sup>6,7</sup> urine,<sup>8</sup> saliva<sup>9</sup> and cerebrospinal fluid,<sup>10</sup> present significant advantages: (1) body fluids are a fresh source of biomarkers; (2) the samples are obtained through non-invasive or minimally-invasive procedures; and (3) liquid samples can be collected at any point throughout the course of the therapy, providing dynamic information regarding the tumor evolution. Nevertheless, all these benefits are hampered by the lack of sensitivity or specificity of most protein cancer biomarkers, such as prostate specific antigen (PSA)<sup>11,12</sup> or carcinoembryonic antigen (CEA).<sup>13</sup> Thus, liquid biopsies for these proteins are only used as supplementary diagnostic tools. In addition, the analysis of cancer biomarkers has been mostly focused on disease diagnosis, rather than prognosis, which could have the potential to improve the treatment and disease management.

During the last few years, increasing amount of research has been published regarding new kinds of circulating biomarkers, such as cell-free and circulating tumor DNA,<sup>14</sup> microRNA,<sup>15</sup> circulating tumor cells<sup>16</sup> and exosomes.<sup>17</sup> They

<sup>a</sup>Biophysics Group, Department of Physics and Astronomy, University College London, London, WC1E 6BT, UK. E-mail: ntk.thanh@ucl.ac.uk

<sup>b</sup>UCL Healthcare Biomagnetic and Nanomaterials Laboratories, 21 Albemarle Street, London W1S 4BS, UK

<sup>c</sup>Institute of Materials Research and Engineering, A\*STAR (Agency for Science, Technology and Research), Innovis, #8-03, 2 Fusionopolis Way, Singapore, 138634. E-mail: xd-su@imre.a-star.edu.sg

<sup>d</sup>Department of Chemistry, National University of Singapore, Block S8, Level 3, 3 Science Drive 3, Singapore, 117543

<sup>e</sup>School of Science and Engineering, University of the Sunshine Coast, Locked Bag 4, Maroochydore DC, Queensland, 4558, Australia

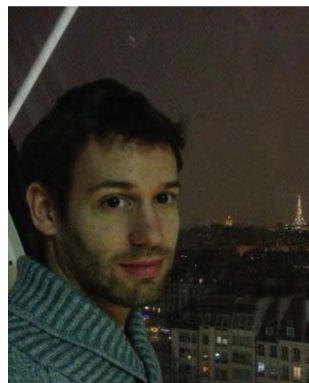


have been identified as potential biomarkers with unique clinical opportunities for diagnosis and prognosis of cancer because of (1) their high stability in body fluids; (2) concentration-dependence of disease states; and (3) rapid clearance from the bloodstream, providing real-time information of the patient condition.<sup>18</sup> However, their applications in the clinic have been limited by low concentration and high heterogeneity (in some cases). New sensing methodologies with high sensitivity, tolerance to complex matrix backgrounds and multiplex detection are required to promote the clinical applications of circulating biomarkers.

Noble metals have been used in medicine throughout the history of civilization.<sup>19,20</sup> For instance, Egyptians were already using gold in dentistry around 4500 years ago,<sup>21</sup> Persians used to stock clean water in silver containers in order to avoid contamination<sup>22</sup> and the Hippocratic Corpus, an ancient medical Greek book written in the 5<sup>th</sup> century B.C., described the use of gold wires in jaw fractures.<sup>23</sup> During the past couple of decades, significant progress in colloidal and surface chemistry has resulted in a significant volume of basic and applied research of noble metals at the nanoscale, such as metal nanoparticles (mNPs).<sup>24–26</sup> The size-dependent physicochemical properties of mNPs have allowed them to be used as sensitive

transducers for bioassays and sensors to detect various biological events through color change, fluorescence modulation and spectroscopy enhancement.<sup>27–29</sup> These extended applications are based on mNPs being easily functionalized by many recognition elements, such as DNA, proteins and antibodies, that provide selectivity towards different biological targets.<sup>27,30,31</sup> The combination of highly versatile nanoparticle sensing principles with recognition elements has resulted in bioassays with fast responses and visual outcome, suitable for use in resource limited environments (Scheme 1).<sup>32</sup>

The great interest surrounding circulating cancer biomarkers for cancer diagnosis has been shown in the publication of numerous reviews focusing on the biology<sup>17,38–40</sup> and clinical aspects of these biomarkers<sup>14,16,41,42</sup> as well as the different techniques used to analyze them,<sup>43–46</sup> including some based on nanomaterials and nanoparticles.<sup>47,48</sup> There is not yet, however, a critical review that (1) critically evaluates the status of circulating cancer biomarker based diagnosis, such as the common techniques used in the analysis and their current limitations, (2) describes how methods based on mNPs can overcome these limitations and (3) discusses what possibilities nanosensing may offer in the coming years. In this review we bridge this gap by analyzing the recent progress



**Roger M. Pallares**

*Lawrence Berkeley National Laboratory (CA, USA). His research interests focus on the use of nanomaterials for biomedical applications.*

*Roger M. Pallares: After working on the growth of 2D nanomaterials at NTT Basic Research Laboratories (Japan), he started a joint doctoral program between University College London (UCL, UK) and the Agency for Science, Technology and Research (Singapore), obtaining a PhD in materials science from UCL in 2016. He has worked as a post-doctoral scholar at Northwestern University (IL, USA), and is currently a postdoctoral fellow at*

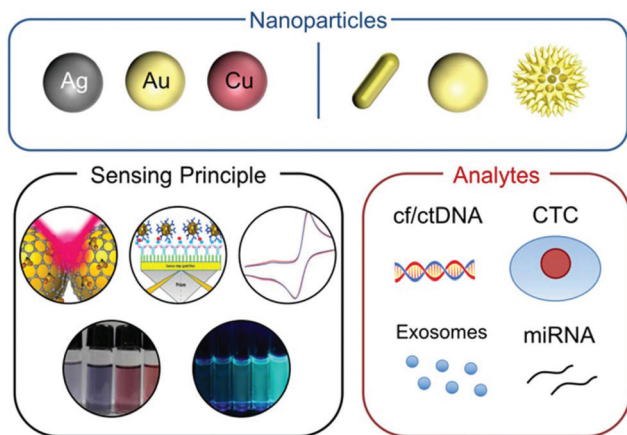


**Nguyen Thi Kim Thanh**

*nanomaterials for biomedical applications. In collaboration with physicists, material scientists, and chemical engineers she has produced the next generation of nanoparticles with very high magnetic moment, fine tuning Au nanorods, and novel hybrid and multifunctional nanostructures. Detailed mechanistic studies of their formation by sophisticated and advanced analysis of the nanostructure allow tuning of the physical properties at the nanoscale; these can subsequently be exploited for diagnosis and treatment of various diseases such as cancers in collaboration with biologists, biochemists and clinicians. She has published over 100 peer reviewed journal articles and book chapters, books and theme issues. Among them 10 papers were featured as cover pages. Her research has accrued over 9000 citations, with an average of 65 citations per item, giving her an i10 index of over 50, and notably she has 12 papers with over 100 citations, with 1 research paper attracting an exceptional over 2300 citations.*

*Professor Nguyen T. K. Thanh, FRSC, FIMMM, FInstP, FRSB (<http://www.ntk-thanh.co.uk>), held a prestigious Royal Society University Research Fellowship (2005–2014) and was promoted to full professor of nanomaterials in 2013 at University College London. She conducts cutting edge interdisciplinary and innovative research on the design, chemical synthesis, and physical characterization of magnetic and plasmonic*





**Scheme 1** Schematic representation of the main components on the sensing of circulating cancer biomarkers by mNPs covered in this review. Adapted with permission from ref. 33 (Copyright 2011 Elsevier), ref. 34 (Copyright 2010 Royal Society of Chemistry), ref. 35 (Copyright 2018 Materials Research Society), ref. 36 (Copyright 2010 American Chemical Society), and ref. 37 (Copyright 2012 American Chemical Society).

in using mNPs for circulating cancer biomarker sensing. We introduce the topic by summarizing the fundamentals of mNPs and their sensing principles; we also describe the clinical opportunities that each circulating cancer biomarker offers. We then evaluate the analytical challenges that MNP-based assays have overcome and further comment on the great potential that mNP-based biosensors hold for the future.

## 2. Metal nanoparticles as sensing materials

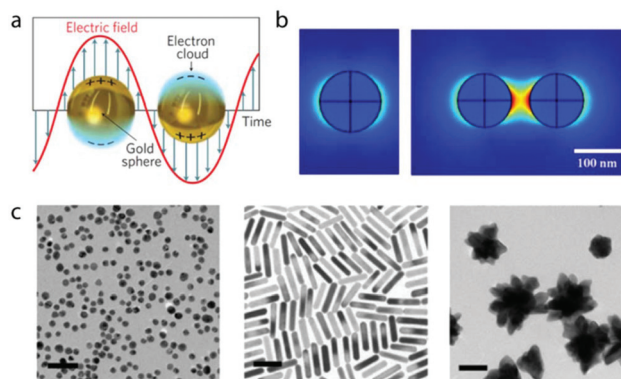
### 2.1. Localized surface plasmon resonance

The unique optical and electronic properties of mNPs are the result of the collective oscillation of the conduction band elec-



**Xiaodi Su**

*Professor Xiaodi Su received her PhD in analytical chemistry from Nankai University (China) in 1995. Currently she is a senior scientist and strategic research office councillor in the Institute of Materials Research and Engineering (IMRE), A\*STAR, Singapore. She is also an adjunct associate professor in the Department of Chemistry at the National University of Singapore. Her research interests include developing advanced analytical technologies and nanomaterial-based biosensors for medical diagnosis, environmental monitoring, and food analysis.*



**Fig. 1** (a) Schematic representation of electron collective oscillation with the incident electromagnetic field in gold nanoparticles. Adapted with permission from ref. 61 (Copyright 2011 Springer Nature). mNPs of other materials, such as silver or copper, also exhibit LSPR. (b) Simulation of the electromagnetic field ( $V m^{-1}$ ) for a single 100 nm gold nanoparticle and a dimer with incident 633 nm wavelength light. Adapted with permission from ref. 62 (Copyright 2011 MDPI). (c) Polymer-coated AuNPs with spherical, rod and star morphologies. Adapted with permission from ref. 63 (Copyright 2010 Royal Society of Chemistry).

trons when perturbed by an external electromagnetic radiation.<sup>49</sup> This electromagnetic force pushes away the electron cloud from the equilibrium position, inducing a surface polarization that promotes the oscillating movement and restores the system's equilibrium (Fig. 1a). This phenomenon occurs at very specific light frequencies and is called localized surface plasmon resonance (LSPR).<sup>50</sup> The first quantitative explanation of LSPR appeared in 1908, when Gustav Mie solved Maxwell's electromagnetic equations for small spherical gold particles.<sup>51</sup> Despite that pioneering work, the unusual optical properties of mNPs did not achieve widespread popularity until two decades ago, when different morphologies were finally accessible through newly developed synthetic protocols.<sup>52,53</sup> The new colloidal chemistry combined with improvement in computing power provided a deeper understanding and refreshed the interest on the interaction between mNPs and light.

LSPR is sensitive to many parameters, such as size and shape of the nanoparticle,<sup>54</sup> refractive index of the surrounding medium,<sup>55</sup> ligands on the surface,<sup>56</sup> temperature,<sup>57</sup> and interparticle distance.<sup>58,59</sup> Depending on the nanoparticle size and shape, the energy of excited plasmons is released by radiative (*i.e.* scattering of light) or non-radiative pathways.<sup>60</sup> The non-radiative paths include the production of heat (if the nanoparticle is isolated) or electron transfer to adsorbed entities, such as in catalysis or semiconductor doping.

Strong far- and near-field effects are produced when the plasmon is excited. The former defines the extinction, scattering and absorption cross section of the nanoparticles. The latter affects the space near the surface, changing the interaction with nearby molecules or other particles. These near-field effects are used in several analytical techniques, such as surface enhanced Raman spectroscopy<sup>64</sup> or fluorescence enhancement.<sup>65</sup> The plasmon fields in nanoparticles are more



sensitive to distance (*i.e.* they scale as  $1/r^3$ , where  $r$  is the distance from the metal surface) than in bulk metal (*i.e.* they scale as  $1/r$ ).<sup>66</sup> This strong distance-dependency confines the electromagnetic field around the nanoparticle, becoming a very localized and high-density phenomenon (Fig. 1b). When two nanoparticles are placed in close proximity (*i.e.* distances below half nanoparticle diameter), their LSPR couples, changing the behavior of the plasmon and producing hot spots in the nanoparticle gap.<sup>67</sup> The stronger electromagnetic field in the gap can be used in different ways, such as enhancing Raman scattering signals, which can achieve single-molecule sensitivity.<sup>68</sup>

## 2.2. Different classes of mNPs

Gold (Au), silver (Ag) and copper (Cu) are attractive candidates for optical technologies because their LSPR bands are in the visible region, in contrast to transition metals, whose plasmon bands lie in the UV region.<sup>69</sup> Nevertheless, since Cu is easily oxidized,<sup>70</sup> most mNP studies have been focused on Au and Ag.

The most common strategy in the synthesis of mNPs is salt-reduction. In this approach, a soluble metal salt is reduced by a reducing agent in the presence of a stabilizing component, which tailors the growth of the crystal in different morphologies, including nanospheres, nanorods, and nanostars (Fig. 1c), and prevents aggregation and precipitation.

*Spherical gold nanoparticles* (AuNPs) are the most commonly used mNPs and are frequently synthesized through the Turkevich method, where  $\text{Au}^{3+}$  salts are reduced to  $\text{Au}^0$  by citrate at 100 °C.<sup>71</sup> Then, the metallic Au nucleates yielding small AuNPs (~2–4 nm), which aggregate, rendering larger particles with diameters around 20 nm. Interestingly, no further stabilization is required since citrate acts as both reducing and capping agents. Further studies proved that the nanoparticle diameter could be adjusted from 20 to 60 nm by changing the molar ratio between  $\text{Au}^{3+}$  and citrate.<sup>72,73</sup> Although the original Turkevich method dates back to 1951, the growth mechanism of the particles is still not fully understood, being the subject of several recent studies.<sup>74–76</sup> Another popular synthesis of AuNPs is the Brust–Schiffrin method, where  $\text{Au}^{3+}$  cations (from  $\text{HAuCl}_4$ ) are reduced by  $\text{NaBH}_4$  in a two-phase (water/toluene) system, in the presence of alkanethiol. The nanoparticles grow in the toluene phase with diameters ranging from 1 to 3 nm.<sup>77</sup> Finally, monodisperse AuNPs have been recently obtained with fast reaction times by taking advantage of microfluidic flow reactors.<sup>78,79</sup>

*Gold nanorods* (AuNRs) are frequently used for biological applications because their tunable longitudinal LSPR band can be easily shifted within the near-infrared biological window by changing the rod aspect ratio. In addition, AuNRs show stronger near-infrared absorption than other morphologies and scatter light at smaller sizes.<sup>54</sup> AuNRs are synthesized through a seed-mediated method where hexadecyltrimethylammonium bromide is used as a directing agent.<sup>80–84</sup>

*Gold nanostars* (AuNSs) are composed of a spherical core and several protruding tips.<sup>85</sup> The optical behavior of AuNSs is due to the combination of the two components, with a small plasmon band originating from the core and a big one from

the tips. Interestingly, the position of the main plasmon band strongly depends on the aspect ratio, aperture angle and roundness of the tips but little on their number.<sup>86</sup> AuNSs are synthesized through both seedless and seed-mediated methods<sup>31,85–87</sup> and their main LSPRs induce strong field enhancements near the tip ends.<sup>88</sup> This strong field has been used to enhance the SERS signal and decrease the detection levels down to the zeptomolar level.<sup>89</sup>

*Spherical silver nanoparticles* (AgNPs) are also grown through the Turkevich method,<sup>90–92</sup> where citrate acts as both reducing agent and ligand.<sup>93</sup> While the Turkevich protocol yields small and spherical AuNPs, this method produces more heterogeneous Ag particles with larger sizes (*i.e.* diameters around 60 nm). Recent advances in their synthesis include the use of microfluidic reactors for continuous growth of the particles.<sup>79,94</sup> AgNPs have bluer LSPR bands than AuNPs and stronger field enhancements.<sup>95</sup> Thus, since the publication of the early synthetic protocols, AgNPs were successfully used as a substrate for SERS.<sup>90,92</sup>

*Metal nanoclusters* (mNCs) are a subclass of mNPs that show luminescence properties. mNCs are made of a few to hundreds of atoms with particle sizes smaller than 2 nm.<sup>96,97</sup> They present discrete electronic states as a result of strong quantum confinement effects<sup>98</sup> (due to the sub-2 nm sizes) and strong interaction with ligands.<sup>99</sup> Thus, mNCs present molecule-like behavior, such as HOMO–LUMO transitions,<sup>100</sup> strong fluorescence<sup>101,102</sup> and quantized charging.<sup>103</sup> These properties make them ideal candidates for catalysis<sup>104–106</sup> and optical technologies.<sup>107,108</sup> Ligand selection in the growth of mNCs is essential to stabilize them and tune the final emission wavelength.<sup>109–114</sup>

## 2.3. Sensing principles for mNP assays

mNPs have been used in many sensing applications because of their ability to absorb and scatter light in the LSPR frequencies, which can be customized in the visible and near infrared regions of the spectrum.<sup>32</sup> The sensing principles are versatile,<sup>115</sup> including colorimetry based on particle aggregation, LSPR exploiting refractive index changes, fluorescence enhancement or quenching caused by nanoparticle energy transfer, and surface enhanced spectroscopy. The nanoparticles with LSPR found in the visible range are ideal probes for rapid on-site sensing application, including point-of-care diagnostics, since the changes of absorption can be detected by the naked eye or using inexpensive instruments.<sup>116</sup> Nanoparticles that absorb or scatter light in the near IR region are more suitable to detect analytes in biological samples because their LSPR is located within the biological optical window, where sample matrix interferences are minimized.<sup>117</sup>

**2.3.1. Colorimetric detection.** Colorimetric assays are based on the inter-particle plasmonic coupling between particles that renders a change in the solution color (*i.e.* from red to blue in the case of AuNPs) as the LSPR band red-shifts because of particle aggregation (Fig. 2).<sup>118</sup> The color change can be induced by any analyte binding that directly or indirectly aggregates (or disaggregates) the nanoparticles, and



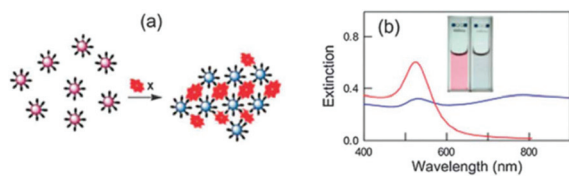


Fig. 2 (a) Scheme of analyte-triggered AuNP aggregation and (b) the corresponding change in the nanoparticle optical properties. Reproduced with permission from ref. 132 (Copyright 2012 Royal Society of Chemistry).

thus serves as a measure of the presence of specific analytes.<sup>119–121</sup>

Early aggregation-based designs date back to the 1990s and were initially focused on the detection of single-stranded oligonucleotides. Two sets of AuNPs were functionalized with two probe strands, and the hybridization between the probes and a target that contained complementary sequences to the two probes triggered the nanoparticle aggregation.<sup>122</sup> Mirkin *et al.* were able to obtain sensitivities down to the femtomolar level with these designs.<sup>122</sup> Functionalization of AuNPs with antibodies allowed the detection of other antigens such as proteins. This was the principle behind the pregnancy tests commercialized by Carter-Wallace in the early/mid-1990s.<sup>123</sup>

Since the early reports of Mirkin *et al.*,<sup>122</sup> aggregation-based assays have become one of the most studied mNP-based designs used to characterize multiple analytes through cross-linking aggregation, such as on-particle DNA hybridization and/or antigen–antibody recognition.<sup>124,125</sup> Later, non-cross-linking ones, which are based on electrostatic interactions, were also developed.<sup>126–129</sup> These aggregation based assays present several key advantages over cross-linking and other homogeneous assays (such as fluorescence-based ones<sup>130,131</sup>), including label-free protocols, fast homogeneous solution interactions and allowing detection by the naked eye or using low cost instruments.<sup>32</sup> Nevertheless, there are still a few issues that hinder the aggregation-based assay applications in a complex matrix, such as variable nanoparticle stability under changes of pH, temperature or ionic strength.<sup>32</sup> The upcoming improvements on particle colloidal stability will define the future applications of mNP aggregation-based designs.

**2.3.2. Refractive index sensing.** Refractive index sensors are based on the change of the dielectric constant of the metal vicinity by the analyte. Because this effect is distance dependent, the analyte has to be located in close proximity to the metal surface to effectively change the refractive index of the surroundings and shift the position of the plasmon band.<sup>133</sup> The first designs of refractive index sensors were developed in the early 1980s and used Ag films as substrates.<sup>134,135</sup> Nowadays, however, most common designs employ a thin Au surface as a plasmonic material and are known as surface plasmon resonance (SPR) sensors.<sup>136</sup> In recent years, several alternatives using AuNPs,<sup>137</sup> Au nanocrosses,<sup>138</sup> Au nano-holes<sup>139</sup> or Ag triangles<sup>140</sup> have been developed in order to improve the technique sensitivity compared to Au films.

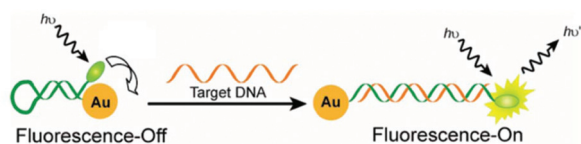
**2.3.3. Surface enhanced Raman spectroscopy.** Raman scattering is the inelastic scattering of electromagnetic radiation by a target molecule.<sup>141</sup> Since this phenomenon depends on the vibrational modes of the molecule, the spectrum is characteristic for each target.<sup>142</sup> Nevertheless, the efficiency of inelastic scattering is low.<sup>143</sup> The intensity of Raman scattering can be enhanced by placing the analyte near a metal surface, whose plasmon field is excited.<sup>143</sup> This approach is called surface enhanced Raman spectroscopy (SERS). Isolated AuNPs have been reported to enhance the signal up to  $10^3$ – $10^4$  and aggregated nanoparticles up to  $10^{15}$ ,<sup>144</sup> which allows single-molecule detection.<sup>145,146</sup> The formation of hot spots between AuNP gaps, where their plasmon fields are coupled, is accounted for by more significant signal enhancements. Interestingly, theoretical studies indicate that field effects on hot spots can only enhance the SERS signal up to  $10^{10}$ .<sup>147</sup> Therefore, an additional factor besides the electromagnetic field has to play a role in the SERS signal generation. Otto and Persson proposed a chemical factor, where the ballistic electrons in the metal interact with a strongly chemisorbed molecule.<sup>148,149</sup> Because direct Raman measurement of analytes within complex samples may yield very complicated SERS spectra, active dyes known as Raman tags are used. Thus, the analytes are detected through indirect assays, where the Raman signals of the tags bound to the analytes are measured.<sup>150</sup>

**2.3.4. Fluorescence-based detection.** Although fluorescence is one of the most well-established techniques in sensing and biomedical diagnostics,<sup>151</sup> there is still a need for improving the sensitivity.<sup>152</sup> There are several factors that limit fluorescence applications, such as photobleaching of fluorophores<sup>153</sup> and autofluorescence of luminescent samples.<sup>154</sup> Modifying the emission behavior of fluorophores by coupling them with a metal surface has been applied to overcome these limitations.<sup>152</sup>

*Plasmon-resonance energy transfer (PRET) sensing.* When a donor (*e.g.* organic dye or quantum dot) is placed near a metal surface, a resonant energy transfer occurs in a similar way to Förster resonance energy transfer (FRET) between organic dyes or quantum dots.<sup>155</sup> In addition to the energy transfer, the plasmon also affects the donor radiative lifetime.<sup>155</sup> Both effects contribute to the strong fluorescence quenching, which can be described by the Gersten–Nitzan model.<sup>156</sup> Even though FRET and PRET have similarities, they also present significant differences: PRET shows stronger quenching efficiency, due to the greater molar extinction coefficient of the plasmonic nanoparticles in comparison with organic dyes.<sup>60</sup> FRET occurs in a distance range from 1 to 10 nm between the donor and acceptor, while nanoparticle-based PRET can double that distance.<sup>157</sup> Photoluminescence and luminescence lifetime experiments have proved that the fluorescence quenching in PRET decreases at a metal–dye separation of  $1/d^4$ , while traditional FRET decreases at a donor–acceptor distance of  $1/d^6$ .<sup>158</sup>

A wide group of PRET sensors have been designed by combining mNPs with different kinds of donors, such as organic dyes,<sup>159,160</sup> quantum dots,<sup>161–163</sup> metal nanoclusters<sup>164,165</sup> and conjugated polyelectrolytes.<sup>166,167</sup> These have been applied for





**Fig. 3** Scheme of a molecular beacon exploiting the PRET principle for DNA sensing. Reproduced with permission from ref. 27 (Copyright 2012 American Chemical Society).

the sensing of metal ions,<sup>159,168</sup> small molecules,<sup>169,170</sup> proteins,<sup>171</sup> and bacteria<sup>172</sup> and for tracking molecular events, such as protein–DNA binding.<sup>166</sup> Interestingly, PRET has been used in molecular beacons for DNA sensing.<sup>173</sup> In this, the extremes of a self-complementary probe with a hairpin structure are functionalized with a donor and a mNP. The hairpin structure locates the donor near the nanoparticle, leading to strong fluorescence quenching. Upon probe hybridization with the target, the hairpin structure opens in a rod-like conformation and separates the donor and the acceptor (Fig. 3). The fluorescence is then restored and the intensity depends on the target concentration. This principle can also be used to monitor the cleavage of nucleotides by nucleases.<sup>174</sup>

**Metal-enhanced fluorescence sensing.** Under some conditions, the electromagnetic coupling between a mNP and a fluorophore yields additional de-excitation pathways, which may enhance the fluorophore's excitation rates and/or the radiative decay rates that in turn result in fluorescence enhancement.<sup>175–177</sup> Both plasmon-induced quenching and fluorescence enhancement compete and are distance dependent phenomena. At short distances, the energy transfer between the fluorophore and plasmon dominates.<sup>178</sup> However, at specific distances farther from the metal surface, the energy transfer is highly reduced, while the electromagnetic field is still strong enough to enhance the fluorescence.<sup>179,180</sup> A distance range between 10 and 20 nm from the metal surface has been reported to present the strongest fluorescence enhancement.<sup>178</sup> Additionally, the plasmon band overlapping the fluorophore's emission and absorbance bands is also required for maximum enhancement.<sup>175</sup> Regarding the role of the size and morphology of nanoparticles, the particles with a larger scattering cross-section present higher fluorescence enhancement.<sup>181,182</sup> Even though metal-enhanced fluorescence sensors are not as common as PRET sensors, some homogeneous assays using this mechanism have been developed for the detection of biomolecules, such as DNA<sup>183</sup> or proteins.<sup>184</sup>

**2.3.5. Electrochemical detection.** The biocompatibility, electronic and catalytic properties of mNPs make them attractive substrates for electrochemical sensing.<sup>185</sup> In these, the nanoparticles play a role in recognizing the analytes as well as generating the electronic signal for sensing.<sup>186</sup> Although mNPs can be used in a wide range of electrochemical schemes, most of them can be classified into two groups: (1) nanoparticle-based enzyme electrodes, where mNPs enhance the electron transfer between the redox centers of proteins, which usually

are insulated by the protein shell, and the electrodes<sup>187</sup> and (2) nanoparticle-based bioaffinity electrodes, where mNPs amplify the transduction of the analyte–electrode interaction through a sandwich immunoassay or another labeling protocol.<sup>187</sup> In addition to these two schemes, mNPs can also be used to immobilize biomolecules and to catalyze electrochemical reactions on the electrodes.<sup>188</sup>

### 3. Analysis of circulating cancer biomarkers with metal nanoparticles

#### 3.1. Cell-free DNA and circulating tumor DNA

The discovery of DNA in blood plasma by Mandel *et al.* dates back to 1948.<sup>189</sup> These extracellular DNA molecules are adsorbed on proteins (histones) and are predominantly around 180 base pair long.<sup>190</sup> Although the exact mechanism that promotes the release of cell-free DNA (cfDNA) into the bloodstream is not fully understood,<sup>191</sup> sequencing analysis indicates that cfDNA originates from apoptotic cells in healthy individuals.<sup>190</sup> In patients with high cell turnover disorders, such as cancer, myocardial infarction and autoimmune conditions, higher levels of cfDNA with both apoptotic and necrotic origins are detected.<sup>193</sup> A meta-analysis on the diagnostic accuracy of cfDNA showed similar results compared to conventional biomarkers and not enough differentiation capabilities to be used as a single cancer indicator.<sup>191</sup> Multiple reports for different types of cancer, however, highlighted the cfDNA prognostic value, where the oligonucleotide concentration levels could be correlated to relapse probability and overall survival.<sup>191</sup> Therefore, cfDNA has been highlighted as a promising biomarker to predict the patient outcome and forecast relapse probability rather than to diagnose.

Between 3% and 93% of all DNA in the bloodstream originate from tumor cells in cancer patients, depending on the stage and size of the tumor.<sup>193,194</sup> This DNA with tumor origin is known as circulating tumor DNA (ctDNA) and contains characteristic genetic alterations identical to those from the tumors that can be targeted for non-invasive diagnosis. For instance, detectable levels of ctDNA were present in more than 75% of pancreatic, ovarian, colorectal, melanoma, breast, gastroesophageal, melanoma, head and neck cancer patients.<sup>195</sup> Furthermore, ctDNA could be observed in patients without other measurable biomarkers, such as circulating tumor cells.<sup>195</sup> Regarding the clinical value, KRAS mutation analysis of ctDNA showed a sensitivity and a specificity of 96 and 95%, respectively, in the diagnosis of thoracic malignancies.<sup>39</sup> ctDNA has also shown greater correlation with changes in tumor burden compared to conventional markers, such as CA 15-3 in metastatic breast cancer patients.<sup>196</sup>

Hence, cfDNA (overall amount of DNA in the bloodstream) is a good biomarker for patient prognosis (*i.e.* forecast of disease outcome), while the levels of ctDNA have shown promising results in diagnosis (*i.e.* identification of type of disease and stage).



Both cfDNA and ctDNA are extracellular oligonucleotides but are not in a completely free form. Because blood is rich in DNases, *i.e.* enzymes that cleave the DNA backbone, only DNA adsorbed on proteins or complexed in lipid structures remains stable in the bloodstream.<sup>40</sup> In order to extract the DNA from these complexes, several commercial kits based on silica membranes have been developed that yield the isolated oligonucleotides in buffer solution.<sup>44</sup>

There are two main strategies to analyze plasma DNA for cancer diagnosis, targeting either cfDNA or ctDNA.<sup>14</sup> For cfDNA, whose total concentration (not the specific sequence) is correlated to the patient prognosis, techniques such as UV-Vis spectroscopy, fluorescent intercalating dyes and quantitative real-time polymerase chain reaction (qPCR) are used.<sup>14</sup> Although these techniques are commercially available, they have limits in their accuracy of detection because the limit of detection is too close to the clinically relevant concentrations, or require complex and time consuming enzymatic amplification. For ctDNA, genetic mutations from the primary tumor are identified and quantified in the plasma DNA. Because there are frequently occurring mutations that drive tumor formation, such as point mutations and deletion mutations in KRAS or EGFR, the ctDNA analysis can target these genetic alterations through digital PCR or next generation sequencing techniques.<sup>197,198</sup> Alternatively, untargeted methods including genome-wide detection of single nucleotide mutations, as well as mutations of larger genome sections, such as rearrangements and chromosomal copy-number, have been developed for general genetic analysis without focusing on specific known mutations.<sup>199,200</sup>

**3.1.1. Quantification of cfDNA.** The majority of mNP-based sensing techniques target ctDNA with specific cancer-related sequences (more discussion in the next section). The demand towards cfDNA quantification, however, has led to the development of several mNP sensors and assays. The detection of cfDNA with clinical impact is challenging because of the wide physiological concentration range (from low ng ml<sup>-1</sup> to high µg ml<sup>-1</sup>) for different cancer types. Hence, an effective sensor must be able to cover as wide as possible concentration ranges. Two strategies have been developed to address this challenge: (1) mNP-based inverse sensitivity response assays<sup>127</sup> and (2) designs based on SERS.<sup>201,202</sup> The first approach exploits the electrostatic interaction between hexadecyltrimethylammonium bromide coated AuNRs and DNA, which results in an unusual DNA concentration-dependent aggregation of the particles.<sup>127,203</sup> The colorimetric assay provides inverse sensitivity, where the lower the analyte concentration, the higher the sensing response, and covers a wide range of cfDNA concentrations (from 20 ng ml<sup>-1</sup> to 10 µg ml<sup>-1</sup>), which are associated with different types of cancer.<sup>127</sup> The second strategy records the SERS spectra of serum cfDNA and identifies characteristic peaks.<sup>201</sup> For instance, Ito *et al.* were able to distinguish with Ag nanoscale column chips individuals with gastric and colorectal cancer from healthy individuals and patients with benign disease by their distinct SERS peak heights.<sup>202</sup>

**3.1.2. Detection of single nucleotide polymorphisms in ctDNA.** Single base variations or single nucleotide polymorphisms (SNPs) are the most common genetic mutations, and are associated with several health disorders including cancer.<sup>204</sup> Because many studies have proved the correlation between these mutations and the clinico-pathological features of multiple types of tumors, single nucleotide polymorphisms are being explored as cancer biomarkers.<sup>205</sup> In order to achieve the sensitivity and specificity required to distinguish one mutation within long ctDNA sequences, most of the assays combined the particle sensing principle with enzymatic amplification techniques. For instance, a common strategy has been amplifying the target sequence with PCR, and using the oligonucleotides to protect AuNPs against salt-induced aggregation.<sup>206–208</sup> The interactions between DNA and the Au surface could be enhanced by using thiolated PCR products.<sup>209</sup> More sophisticated methods combined PCR-amplified targets with additional enzymes, such as ligases, where only perfectly matched targets and probes were ligated inducing permanent particle aggregation (Fig. 4).<sup>210</sup> The sensitivity of the ligase reaction assay could be improved down to 1 pM by attaching one probe to the Au surface and using SPR spectroscopy.<sup>192</sup> Although PCR amplification is the most common protocol before the ligase detection reaction, target amplification could be eliminated for SNP detection by combining ligases and two primers, one containing a Raman tag and the other a AuNP acting as a Raman enhancer.<sup>211,212</sup> Beyond PCR and ligase detection reaction, other enzymatic amplification reactions, including the ligase chain reaction,<sup>213,214</sup> helicase-dependent isothermal amplification,<sup>215</sup> and strand displacement amplification,<sup>216</sup> have been coupled with mNP-based assays with limits of detection in the pM range. It is worth mentioning that a recent publication developed a colorimetric assay based on an enzyme-free click chemical ligation reaction capable of reaching a limit of detection as low as 50 zM.<sup>217</sup> Table 1 summarizes the current progress on SNP detection by mNP-based assays with enzymatic reactions.

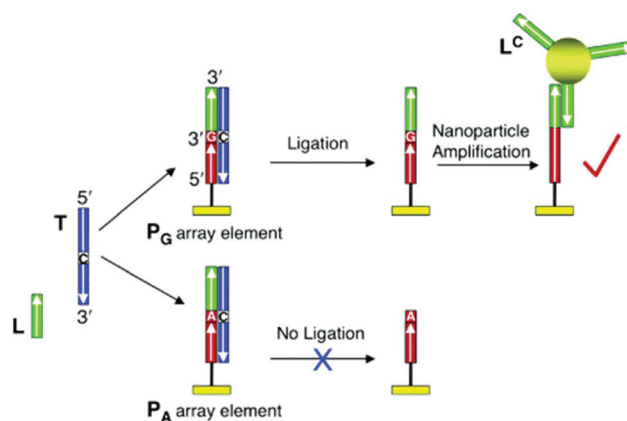


Fig. 4 Scheme of SNP identification based on the combination of a ligase enzyme and nanoparticle-enhanced SPR. Reproduced with permission from ref. 192 (Copyright 2006 American Chemical Society).





**Table 1** mNP-based assays with enzymatic reactions for SNP detection in ctDNA

Type of MNP	Sensing principle	Enzymatic reaction	Gene of SNP	Limit of detection (LOD)	Ref.
Unmodified AuNP	Colorimetric	PCR	XXYLT1	5 fM	206
Unmodified AuNP	Colorimetric	PCR	EGFR	—	207
Unmodified AuNP	Colorimetric	PCR	KCNE1	—	208
AuNPs with thiolated PCR products	Colorimetric	PCR	BRCA1	—	209
ssDNA-AuNPs	Colorimetric	PCR + ligation reaction	KRAS	74 pM	210
ssDNA-AuNPs	SPR spectroscopy	PCR + ligation reaction	BRCA1	1 pM	192
ssDNA-AuNPs	SERS	Ligase detection reaction	KRAS	20 pM	211
ssDNA-AuNPs and ssDNA-AgNPs	SERS	Ligase detection reaction	KRAS	10 pM	212
ssDNA-AuNPs	Colorimetric	Ligase chain reaction	KRAS	20 pg	213
ssDNA-AuNPs	Electrochemical	Ligase chain reaction	p53	0.9 pM	214
ssDNA-AuNPs	Colorimetric	Helicase-dependent isothermal amplification	KRAS	20 pM	215
ssDNA-AuNPs	SERS	Strand displacement amplification	KRAS	1.4 pM	216
ssDNA-AuNPs	Colorimetric	Enzyme-free click chemical ligation reaction	HAV	50 zM	217

**3.1.3. Analysis of copy-number variations, chromosomal translocations, and DNA methylation in ctDNA.** Copy-number variations are types of structural variations, where sections of DNA (ranging from two base pairs up to entire genes) are amplified or deleted, and can contribute to tumorigenesis.<sup>218</sup> Comparative genomic hybridization is a technique developed to survey DNA copy-number variations, where the DNA target and a reference sequence are labelled with dyes, and the fluorescence intensities along the DNA sequences are compared.<sup>219</sup> Mirkin *et al.* were the first to adopt this approach for DNA detection in scanometric arrays, where the fluorescent dye labels were replaced with AuNPs.<sup>220</sup> By introducing Ag deposition following the hybridization of AuNPs with the arrays containing the DNA analyte, the signal intensity could be increased by  $10^5$  fold. Based on this sensor design, Northbrook (IL, USA) has developed a mNP-based technology capable of genotyping DNA in 300 to 500 base fragments.<sup>221,222</sup>

Chromosomal translocations are another type of gene mutation associated with tumors, where parts of the chromosomes are rearranged.<sup>223</sup> The standard techniques to screen these genetic abnormalities are PCR and agarose gel electrophoresis with ethidium bromide staining.<sup>224,225</sup> Kalogianni *et al.* developed a AuNP-based colorimetric lateral flow assay for chromosomal translocations that showed 10-fold higher detectability than agarose gel electrophoresis without the need for special instrumentation.<sup>226</sup> Using this lateral flow assay, they were able to screen seven chromosomal translocations associated with different types of leukemia.

Another promising biomarker for the early detection of malignancy is hypermethylation of ctDNA, since it is an early biochemical event of tumorigenesis.<sup>227</sup> Colorimetric and SERS assays have been developed to simultaneously detect SNP and epigenetic methylations on ctDNA based on AuNPs and enzymatic reactions.<sup>228,229</sup> Alternatively, Zhang *et al.* combined a hairpin DNA probe containing a quantum dot and a AuNP with the bisulfite reaction (*i.e.* a conventional technique used to identify DNA methylation) for the electrochemiluminescence detection of methylation levels and position in ctDNA.<sup>230</sup>

**3.1.4. Current challenges in ctDNA sensing and diagnostics.** Although there are extensive studies highlighting the potential of ctDNA in cancer diagnostics, currently available mNP-based assays are mostly focused only on the detection of SNPs. Many tumors, however, lack recurring DNA mutations and require technologies capable of identifying other cancer signatures through genome-wide analyses.<sup>14</sup> Only a small amount of mNP assays has been developed with genome-wide characterization capabilities and future efforts should be pursued towards that end. There is also a lack of assays that can detect deletion mutation in ctDNA, except one most recent study.<sup>231</sup> Furthermore, a vast majority of the assays still rely on enzyme amplification reactions, which introduce biases due to polymerase and ligase error rates.<sup>14</sup> Designs that use techniques with high sensitivity that do not require enzyme amplification, such as SERS, offer unique opportunities in this regard.

### 3.2. microRNA

microRNAs (miRNAs) are endogenous short RNA sequences (between 21 to 25 nucleotides) that regulate gene expression by translational repression.<sup>38,232</sup> Over 1000 miRNAs have been identified in humans,<sup>233</sup> which participate in a wide range of regulatory processes, such as cell proliferation and tissue growth,<sup>234</sup> apoptosis,<sup>235</sup> developmental timing<sup>236</sup> and haematopoiesis.<sup>237</sup> Hence, aberrant expression of miRNAs has been linked to the onset and progression of multiple pathologies, including cancer,<sup>238,239</sup> central nervous system diseases,<sup>240</sup> metabolic disorders<sup>241</sup> and kidney<sup>242</sup> and liver failures.<sup>243</sup>

Because abnormal miRNA expression profiles can be correlated to the type of tumor and stage, miRNAs are being studied for cancer diagnostics.<sup>244,245</sup> For instance, underexpression of miR-26, miR-143 and miR-145, and overexpression of miR-21 have been observed in multiple cancers, such as colorectal and pituitary adenomas.<sup>246</sup> Furthermore, miRNAs are very stable in blood because of their adsorption on proteins and encapsulation in membrane-bound vesicles, which provide high protection against blood RNases.<sup>247–249</sup> Prior to analysis, however, miRNAs need to be isolated and enriched.<sup>250</sup> Similar to normal total RNA isolation, commer-

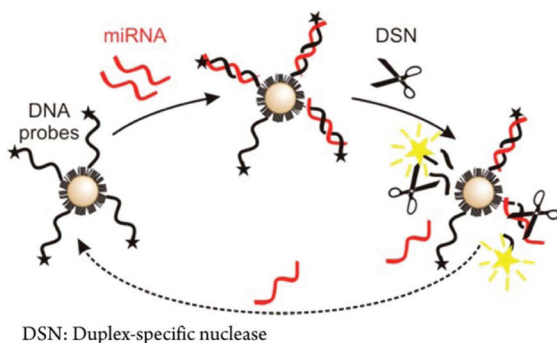


cially available products based on chaotropic salts and solid-phase extraction on silica columns are commonly used.

Northern blotting, microarrays and qPCR are the common methods to detect and quantify miRNAs through oligonucleotide hybridization.<sup>233</sup> However, these methods present several challenges: (1) northern blotting requires large amounts of RNA as a starting material and usually is not sensitive enough to detect less abundant miRNAs,<sup>246</sup> (2) microarrays show lower dynamic range and sensitivities than the other two methods,<sup>233</sup> and (3) miRNA detection by qPCR employs short primers, which result in very low melting temperatures that hamper oligonucleotide replication and quantification.<sup>251</sup>

**3.2.1. Detection of miRNAs.** Similar to DNA detection, miRNAs can be analyzed through mNP-based techniques. The fluorescence recovery of a dye-labelled probe upon hybridization with a target miRNA, such as PRET, is one of the most common systems. In these designs the hairpin-structured probe is assembled on AuNPs. Because the dye is in close proximity to the particle surface, the fluorescence is quenched. Upon hybridization with the miRNA target, the conformation of the probe changes and promotes the fluorescence recovery. This PRET design achieved a LOD of 0.01 pM for miR-122 extracted from liver cancer cells.<sup>252</sup> In addition to fluorescent dyes, other emitters, such as metal nanoclusters, have also been used.<sup>253</sup> The sensitivities of these conformation-switching based PRET assays have been further improved by coupling them to enzymatic reactions, where LODs as low as 200 and 2 aM have been reported for the dye (Fig. 5)<sup>254</sup> and the metal nanocluster-based assays,<sup>255</sup> respectively.

Metal nanoclusters have also been used in other sensing designs beyond fluorescence-based ones. For instance, oligonucleotide encapsulated Ag nanoclusters were immobilized on Au electrodes and used as electrochemical probes for miRNA detection with a LOD of 67 fM.<sup>256</sup> This assay was based on both sequence recognition for miRNA hybridization and AgNC catalyzing H<sub>2</sub>O<sub>2</sub> reduction. More common electrochemical approaches involved sandwich-type assays between Au or carbon electrodes, miRNA target and functionalized AuNPs.<sup>257–260</sup> The presence of AuNPs amplified the sensor voltammetric signals through a catalytic reaction, which achieved



**Fig. 5** Scheme of a conformation-switching assay coupled with an enzymatic reaction for miRNA analysis. Reproduced with permission from ref. 254 (Copyright 2006 American Chemical Society).

LODs between 10 and 60 fM depending on the AuNP functionalization and type of electrode. The particles can also be used to enhance the signal transduction between the electrode and different enzymes, such as phosphatases<sup>261</sup> and peroxidases.<sup>262</sup>

Although not as common as the PRET techniques, SERS and colorimetric assays have also been used for miRNA sensing. Driskell *et al.* demonstrated that several miRNAs could be detected and distinguished through their unique SERS fingerprints on silver nanorod (AgNR) modified substrates.<sup>263</sup> Based on this concept, a study reported distinct features in the serum miRNA spectra of healthy individuals and colorectal cancer patients. Using AgNP aggregates as substrates the authors could distinguish both populations with a sensitivity and a specificity of 89 and 96%, respectively.<sup>264</sup> Regarding colorimetric assays, only a few studies have been published over the years. Because the detection levels required are very low, traditional AuNP aggregation-based designs were not sensitive enough. They required signal amplification techniques, such as isothermal exponential amplification reaction<sup>265</sup> (*i.e.* an enzymatic reaction with DNA polymerase), strand displacement amplification,<sup>266</sup> or Ag<sup>0</sup> deposition enhancement.<sup>267</sup> Alternatively, the peroxidase-like property of Cu nanoclusters has been used for the colorimetric detection of miRNAs, reaching a LOD of 0.6 pM.<sup>268</sup>

All the previous assays were able to detect a specific target sequence; however, they did not allow the profiling of samples with multiple miRNAs. To address this challenge, a few platforms based on high-density oligonucleotide microarrays have been developed. Fang *et al.* demonstrated a multiplexing design for the detection of hepatic miRNAs built on a SPR imaging array.<sup>269</sup> This approach took advantage of the AuNP-enhanced SPR imaging measurements (through a sandwich assay) and polymerase amplification reaction to achieve a LOD of 10 fM. Furthermore, this method used locked nucleic acids (LNAs) as capturing probes, which show stronger binding affinity with miRNAs than conventional oligonucleotides and have been reported to increase the sensitivity of assays tenfold.<sup>270</sup> Mirkin *et al.* designed a scanometric microarray based on particle light scattering capable of characterizing body fluid samples.<sup>271</sup> The assay took advantage of the deposition of Au<sup>0</sup> onto the immobilized AuNPs to amplify the sensor response and decrease the LOD down to 1 fM.

**3.2.2. Future prospects and challenges of miRNA sensors.** To address the challenges of quantification of multiple miRNAs for diagnosis and prognosis of tumours,<sup>246</sup> future mNP-based assays must provide multiplex capabilities. Specifically, even after isolation, miRNAs are still mixed with different RNAs, such as siRNAs or other non-cancer related miRNAs. Therefore, high specificity is required to identify the targeted sequences. The concentrations of these cancer-related miRNAs are between atto- and nanomolar ranges, and the LOD of the assays should reach these low concentration levels. Taking into consideration the current status of the field, mNP-based assays should emphasize the following aspects: (1) High-density oligonucleotide microarrays have been the only



mNP-based designs capable of profiling efficiently samples containing multiple miRNAs. Further studies should consider combining microarrays with the different sensing techniques available (e.g. SERS, SPR, light scattering or fluorescence, among others). (2) The analytical designs that used locked nucleic acid recognition or signal amplification enzymes showed enhanced specificity, which future studies could benefit from. (3) Due to the small amount of miRNAs in body fluids, techniques that offer low concentration or even single molecule detection, such as SERS, should be further developed.

### 3.3. Circulating tumor cells

Circulating tumor cells (CTCs) are shed from primary tumors during their early formation and growth, and released to the bloodstream.<sup>16</sup> Scientific reports have highlighted that CTC levels can be used to predict the prognosis of metastatic patients.<sup>272,273</sup> As an example, the Food and Drug Administration (FDA) has approved the CTC assays for metastatic castration-resistant prostate cancer (CRPC) evaluation, since these assays present the highest accuracy in the prediction of overall survival in CRPC patients.<sup>274</sup> Furthermore, metastasis-initiating cells (MICs) have been identified within CTC populations of breast<sup>275</sup> and renal cancers.<sup>276</sup> These MICs have been later used in xenograft studies to confirm that they give rise to metastasis in other organs. Therefore, the presence and levels of CTCs can provide key information regarding the patient condition, such as cancer aggressiveness, metastatic burden and relapse.<sup>277</sup>

The analysis and quantification of CTCs are challenging due to their low concentration in body fluids, *i.e.* a few CTCs are found in a background of millions of blood cells in blood.<sup>16</sup> Therefore, most CTC samples are enriched before or during their quantification through biological or physical-based techniques. Biological techniques initially capture the CTCs through biological interactions, such as antigen-antibody interaction, and then isolate them. Magnetic separation,<sup>278–280</sup> where the antibody is coupled to a magnetic bead, or microfluidic devices are the main techniques used for cell separation.<sup>281–283</sup> Sub-populations of CTCs, such as epithelial cell adhesion molecule (EpCAM)-positive cancer cells, have also been enriched by electrical responsive conducting polymers and anti-EpCAM antibodies.<sup>284,285</sup> Physical-based techniques are label-free and exploit physical properties through size-based filtration or centrifugation.<sup>286,287</sup>

After the cells have been captured and separated, they need to be identified. CTCs are the result of heterogeneous processes, which can manifest in different ways and vary from cell to cell.<sup>288–290</sup> Therefore, CTC distinction from normal cells is challenging. Conventional sensing techniques can be classified based on whether they target the cell phenotype or genotype.

In *phenotype detection*, CTCs are detected based on their physical properties, such as buoyant density<sup>291–293</sup> and increased size,<sup>294–296</sup> or their distinctive cell surface antigens. EpCAM is the most common target, since this protein is

expressed in all cells of epithelial origin, but not in blood cells.<sup>43</sup> The FDA-approved CellSearch system (Veridex, LLC, Raritan, NJ, USA) is a standardized technology that captures CTCs with anti-EpCAM antibody-loaded ferrofluids and visualizes them through immunostaining.<sup>297</sup> Nevertheless, this technique presents low sensitivity, since only a small percentage of metastatic cancer patients score positive.<sup>298</sup>

In *genotype detection*, CTCs are collected and enriched and their DNA is subsequently extracted and analysed.<sup>299</sup> Nevertheless, it has been reported that ctDNA presents greater mutation detectability over DNA extracted from CTCs.<sup>39</sup> Thus, the genotype analysis of CTCs is not a common protocol, and ctDNA analysis is preferred.<sup>40</sup>

Although CTCs have been the subject of extensive research, both their low concentration in body fluids and their high heterogeneity are still limiting their clinical applications. Interestingly, mNPs present unique features that can overcome these limitations, such as large surface-to-volume ratios that allow the attachment of multiple recognition elements for enhanced specificity in CTC recognition.

**3.3.1. Detection of the CTC phenotype.** Most mNP-based sensing techniques target the CTC phenotype, where mNPs are functionalized with antibodies or aptamers targeting specific surface analytes. Recent advances in the *systematic evolution of ligands by exponential enrichment* (SELEX), *i.e.* a synthetic technique that generates aptamers by an *in vitro* selection process, have extended the range of targets to complex mixtures of proteins in living cell surfaces.<sup>300</sup> Thus, new aptamers have been discovered against previously unidentifiable CTC-surface analytes.

AuNPs with different morphologies have been used for SERS-based sensing of CTCs. These nanoparticles are dispersed in solution<sup>301</sup> or adsorbed on substrates, such as single-walled carbon nanotubes (SWCNTs).<sup>302</sup> When placed on SWCNTs, the AuNPs are able to produce hot spots, decreasing the LOD down to 10 cells per ml due to field-enhancement effects.<sup>302</sup> Furthermore, the SWCNT-AuNP hybrids have been used as dual systems for sensing and photothermal therapy applications.<sup>302–304</sup> Nevertheless, these assays lack the separation steps and they have been solely applied to samples in buffer.

So far, several SERS-based bioassays compatible with a complex medium have been published. Sha *et al.* were able to detect as low as 50 SKBR3 cancer cells per ml in whole blood by combining functionalized AuNPs with magnetic beads.<sup>305</sup> Lung cancer cells have also been detected in whole blood (LOD of 34 cells per ml) by integrating AuNPs and a low cost CTC-capture substrate made of a nitrocellulose membrane.<sup>306</sup> Alternatively, AuNPs can also be functionalized with proteins or biomolecules, yielding probes that bind to the cancer cell surface through surface receptor recognition. As an example, carcinoma cells overexpress the cell-surface epidermal growth factor receptor (EGFR). Thus, AuNPs functionalized with the epidermal growth factor have been employed in a SERS-based assay to detect squamous cell carcinoma in the peripheral blood of 19 patients.<sup>307</sup> Folic acid (FA) has also been used in the detection of CTCs through SERS assays, since several



cancer types (e.g. ovarian, brain, kidney, breast, lung, cervical and nasopharyngeal) overexpress folate receptor  $\alpha$ . FA-functionalized AuNPs have been reported to detect as low as 5 CTCs per ml in rabbit blood.<sup>308</sup> Both AuNPs and magnetic nanoparticles functionalized with FA have also been combined for magnetic enrichment and SERS-based detection of HeLa cells in rat blood.<sup>309</sup> Recently, this protocol has been applied for the detection of CTCs from cervical cancer in the blood of two first-stage clinical patients.<sup>310</sup>

mNP-based colorimetric assays have also been developed to detect and quantify CTCs. These assays are based on the analyte-triggered aggregation or dispersion of mNPs and the corresponding changes in mNP optical properties. AuNPs were conjugated with different aptamers that targeted proteins overexpressed on cancer cell membranes, such as nucleolin.<sup>311,312</sup> If AuNPs contained aptamers and antibodies (double-targeting), the sensitivity could be increased to 100 CTCs per ml in buffer.<sup>313</sup> In order to expand the colorimetric assay to whole blood samples, a lateral flow device was developed that allowed the detection of 4000 CTCs by the naked eye and 800 CTCs using a spectrometer in a 15  $\mu$ L sample. Alternatively, a chemiluminescent assay was designed, where aptamer-functionalized AuNPs catalyzed the reaction between luminol and H<sub>2</sub>O<sub>2</sub>. This assay could detect 30 cells in 3  $\mu$ L of blood sample.<sup>314</sup> Real-time detection of CTCs *in vivo* has been achieved by photoacoustic flow cytometry.<sup>315</sup> Magnetic nanoparticles were used for separating cells from blood and FA-targeting AuNPs on CNTs as photoacoustic contrast agents.

**3.3.2. Detection of the CTC genotype.** Mirkin *et al.* have developed Au nanoFlares for the detection of messenger RNA (mRNA) for a target gene (Fig. 6).<sup>316</sup> The nanoFlares are spherical AuNPs chemically functionalized with ssDNA, which is complementary to the target mRNA. The ssDNA is pre-hybridized with a shorter oligonucleotide labelled with a fluorescent dye, which is quenched by the AuNP. When the mRNA binds to the recognition sequence, the labelled oligonucleotide is released and the fluorescence increases. Au nanoFlares interact with scavenger receptors in the cell membrane, triggering their cellular uptake.<sup>317</sup> NanoFlare technology has been used for the isolation and detection of live CTCs from whole blood.<sup>318</sup> Furthermore, these AuNPs have also been applied to the spatiotemporal location of a target mRNA in living cells,<sup>319</sup> and attempted to be used for the analysis of different transcripts in several cancer cell lines.<sup>352</sup>

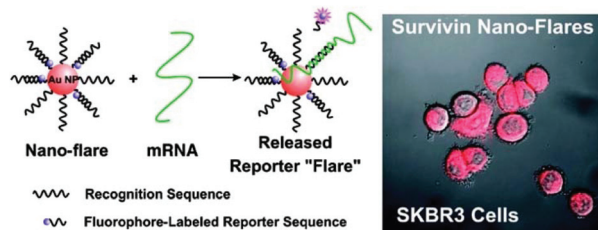


Fig. 6 Au nanoFlare detection of mRNA expressed in CTCs. Reproduced with permission from ref. 316 (Copyright 2007 American Chemical Society).

**3.3.3. Cancer stem cell detection and quantification.** Although CTCs are a heterogeneous group of cells released by the tumors into the bloodstream, there is a specific type that has received increasing attention in recent years. Cancer stem cells (CSCs) are a sub-category of CTCs that share similarities to normal stem cell capabilities, including proliferation and differentiation.<sup>320</sup> Hence, CSCs have been identified as a precursor of metastases and relapses in cancer patients. Antibody functionalized mNPs have been used in the detection of the CSC phenotype through both SERS and colorimetric principles in PBS buffer.<sup>321,322</sup>

### 3.4. Exosomes

Exosomes are vesicles released by cells during the fusion of multivesicular bodies with plasma membranes.<sup>323</sup> The vesicles have diameters between 50 and 90 nm and participate in the regulation of cell waste disposal, intercellular communication and coagulation.<sup>324</sup> Exosomes offer unique opportunities as circulating cancer biomarkers because they contain proteins, DNA and RNA,<sup>248,325</sup> and can be found in multiple biological fluids, such as saliva, blood, serum, urine and breast milk.<sup>326–328</sup>

In order to obtain accurate biological information, exosomes have to be concentrated and isolated from components with similar sizes also found in biological fluids, such as protein complexes, calcium-phosphate precipitates and other lipid vesicles.<sup>329–332</sup> The common protocols for exosome concentration include multi-step ultracentrifugation with speeds up to  $2 \times 10^5 g$ .<sup>333</sup> The recovery yields are low (5 to 25%) and the samples are rich in contaminants, such as protein aggregates, which can lead to wrong biological diagnosis.<sup>334</sup> Furthermore, the whole concentration process is time consuming (4 to 6 h). In order to improve the detection sensitivity and decrease the number of false-negative results, isolation protocols consisting of microfluidics<sup>335,336</sup> and/or immunoaffinity separations are performed.<sup>337,338</sup>

The detection of isolated and concentrated exosomes is still very challenging since these vesicles are very small and highly heterogeneous.<sup>339</sup> Similar to CTCs, traditional detection methods can be classified based on whether they target the phenotype or the genotype.

For detection based on the phenotype, flow cytometry is the gold standard technique to characterize exosomes and other vesicles in clinical samples.<sup>340</sup> A large percentage of the vesicles, however, are smaller than the detection limit of conventional flow cytometers, and modifications, such as labelling with membrane intercalating dyes, are necessary to measure most of the sample contents.<sup>341</sup>

Alternative techniques based on light scattering, such as dynamic light scattering and nanoparticle tracking analysis, can measure the absolute and relative size distributions of exosomes but cannot distinguish them from other particles with similar sizes.<sup>339</sup> Fluorescence-based techniques, including stimulated emission depletion microscopy and fluorescence correlation spectroscopy, can provide both size distributions and biochemical information if antibody labelling is used.<sup>339</sup>

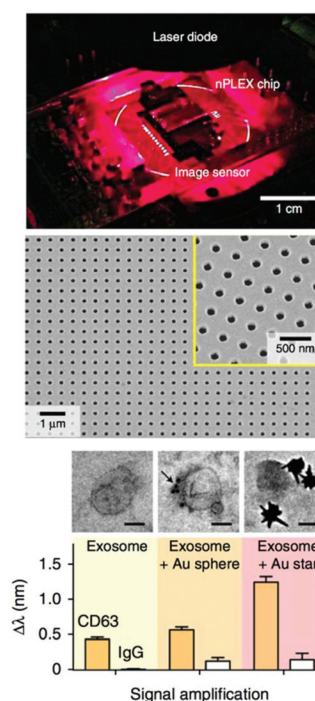


For detection based on the genotype, the DNA and RNA contained in exosomes are isolated and analyzed similarly to ctDNA and miRNA<sup>46</sup> (please refer to sections 3.1 and 3.2 of this review).

**3.4.1. Detection of exosomes.** The strong near-field enhancements of mNPs have been used to study the biological composition of exosomes through SERS assays. For instance, AgNPs adsorbed on silicon micropillars<sup>342</sup> and AuNP aggregates<sup>343</sup> have been used as hot spots in SERS substrates to distinguish between exosomes released by healthy and tumor cells based on the vesicle membrane composition. The magnetic properties of magnetic nanobeads covered by a Au shell have been exploited for both exosome separation and particle aggregation (to generate hot spots) during SERS detection.<sup>344</sup> If the exosomes were dried prior to analysis, additional SERS peaks developed as a consequence of the rupture of the vesicle membranes, exposing the internal content of the exosome.<sup>345</sup> Alternatively, different types of assays have targeted surface antigens to quantify and characterize exosomes, since surface proteins are fundamental for the vesicle biological functions and can provide tumor fingerprints. For instance, the electro-oxidation of AgNPs and copper (Cu) NPs by Au electrodes was used to detect exosomes through an electrochemical assay.<sup>346</sup> The NPs and electrodes were functionalized with anti-EpCAM and anti-PSMA aptamers for multiplex detection and a LOD of 50 exosomes per sensing chip was achieved.

mNPs are not only being used as sensing materials, but are also used for signal amplification of other sensing technologies. For example, AuNPs were coupled with a SPR Au film to detect multiple myeloma-originated exosomes<sup>348</sup> through a structural analogue of heparin sulfate that mediates in exosome endocytosis. In a sandwich arrangement, AuNPs bind to the exosomes, which then are captured on the SPR chip. The addition of AuNPs as signal amplifiers improved the LOD down to tens of pM. A recent study by Im *et al.* demonstrated that if the SPR film was replaced with a periodic Au nanohole array film functionalized with antibodies, and then combined with AuNPs as signal amplification elements, 3000 exosomes derived from ovarian cancer cells (LOD of 670 aM) could be detected (Fig. 7).<sup>347</sup> In this design, the use of 10 nm AuNPs or 50 nm AuNSs improved the signal by 20 and 300%, respectively. The nanohole-based sensor benefited from a significant advantage over traditional SPR platforms: the small array size necessary (below 10 mm<sup>2</sup>) to achieve large optical transmission allowed a high array density (more than 106 detection sites per cm<sup>2</sup>). In addition to the detection and quantification of vesicles, antibody-functionalized AuNPs have been employed to label exosomes for morphology characterization through electron microscopy.<sup>349–351</sup>

**3.4.2. Current challenges on exosome-based diagnostics.** Although promising results have been achieved in the field of cancer diagnostics using tumor-derived exosomes,<sup>45</sup> there is still a lack of understanding of their biology. Over the last decade, multiple studies revealed that these vesicles participate in most tumor-promoting pathways, such as angiogenesis, cancer stemness and hypoxia-induced epithelial–mesenchymal



**Fig. 7** SPR design based on Au nanohole arrays for CTC analysis (top image). SEM image of a Au nanohole array (central image). Effect of nanoparticle labelling on the SPR wavelength (bottom image). Adapted with permission from ref. 347 (Copyright 2014 Springer Nature).

transition.<sup>17</sup> Nevertheless, these new discoveries seem to have only scratched the surface as new work is constantly being published. Further efforts need to be pursued towards establishing and validating the specificity and sensitivity requirements of exosome-based analysis for multiple populations and types of cancers. Only after these requirements have been clearly identified, the clinical utility of exosomes in comparison with other circulating biomarkers will be established.

## 4. Summary and outlook

Circulating cancer biomarker analysis shows promise in the early diagnosis and prognosis of cancer. The clinical application of these biomarkers, nevertheless, is still limited by their low concentrations in body fluids and heterogeneity. Analytical tools capable of achieving high sensitivities and low LOD (below nM in many cases) are necessary for taking advantage of all the information that circulating cancer biomarkers provide regarding the patient condition.

In this comprehensive review, we summarize the current progress on mNP-based analytical methods for the sensing of circulating cancer biomarkers, including cfDNA, ctDNA, miRNAs, CTCs and exosomes. The sensing principles include those where mNPs are used as sensing/transducing materials, such as aggregation induced colorimetric assays, PRET, SERS, and refractive index sensing, as well as those where mNPs are



used as signal amplification materials, for example, SPR and electrochemical sensors. We evaluate the advantages and limitations of each analytical design based on the type of analyte detected, sensing principle, sensitivity and sample preparation. In order to understand the possible impact of mNPs on cancer diagnosis, we analyze the deficiencies of the standard techniques used to detect cancer biomarkers, such as the need for low LOD, ability to work with a complex sample matrix and multiplex detection, and how mNP-based assays can overcome these. Finally, we also revise the current challenges and promising directions of sensing with mNPs. By systematically evaluating the nano- and bioassays, we believe our review will help to inform the current status of the field and to identify future research opportunities.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

N. T. K. T. thanks the EPSRC (EP/M018016/1 and EP/M015157/1) for funding and R. M. P. thanks UCL and the ARAP program of A\*STAR for the scholarship and facilities.

## Notes and references

- M. Gerlinger, A. J. Rowan, S. Horswell, J. Larkin, D. Endesfelder, E. Gronroos, P. Martinez, N. Matthews, A. Stewart, P. Tarpey, I. Varela, B. Phillimore, S. Begum, N. Q. McDonald, A. Butler, D. Jones, K. Raine, C. Latimer, C. R. Santos, M. Nohadani, A. C. Eklund, B. Spencer-Dene, G. Clark, L. Pickering, G. Stamp, M. Gore, Z. Szallasi, J. Downward, P. A. Futreal and C. Swanton, *N. Engl. J. Med.*, 2012, **366**, 883–892.
- L. A. Diaz and A. Bardelli, *J. Clin. Oncol.*, 2014, **32**, 579–586.
- E. G. Robertson and G. Baxter, *Clin. Radiol.*, 2011, **66**, 1007–1014.
- M. J. Overman, J. Modak, S. Kopetz, R. Murthy, J. C. Yao, M. E. Hicks, J. L. Abbruzzese and A. L. Tam, *J. Clin. Oncol.*, 2013, **31**, 17–22.
- M. C. Chamberlain, *Curr. Treat. Options Neurol.*, 2004, **6**, 297–305.
- L. A. Liotta, M. Ferrari and E. Petricoin, *Nature*, 2003, **425**, 905.
- E. F. Petricoin, C. Belluco, R. P. Araujo and L. A. Liotta, *Nat. Rev. Cancer*, 2006, **6**, 961–967.
- J. Nilsson, J. Skog, A. Nordstrand, V. Baranov, L. Mincheva-Nilsson, X. O. Breakefield and A. Widmark, *Br. J. Cancer*, 2009, **100**, 1603–1607.
- N. Raskin, N. A. Babu and K. M. K. Masthan, *Biomed. Pharmacol. J.*, 2015, **8SE**, 701–704.
- M. J. Glantz, B. F. Cole, L. K. Glantz, J. Cobb, P. Mills, A. Lekos, B. C. Walters and L. D. Recht, *Cancer*, 1998, **82**, 733–739.
- I. M. Thompson, D. K. Pauler, P. J. Goodman, C. M. Tangen, M. S. Lucia, H. L. Parnes, L. M. Minasian, L. G. Ford, S. M. Lippman, E. D. Crawford, J. J. Crowley and C. A. Coltman, *N. Engl. J. Med.*, 2004, **350**, 2239–2246.
- J. R. Prensner, M. A. Rubin, J. T. Wei and A. M. Chinnaiyan, *Sci. Transl. Med.*, 2012, **4**, 127rv3.
- E. Björnsson, A. Kilander and R. Olsson, *Liver*, 1999, **19**, 501–508.
- E. Heitzer, P. Ulz and J. B. Geigl, *Clin. Chem.*, 2015, **61**, 112–123.
- P. S. Mitchell, R. K. Parkin, E. M. Kroh, B. R. Fritz, S. K. Wyman, E. L. Pogossova-Agadjanyan, A. Peterson, J. Noteboom, K. C. O'Briant, A. Allen, D. W. Lin, N. Urban, C. W. Drescher, B. S. Knudsen, D. L. Stirewalt, R. Gentleman, R. L. Vessella, P. S. Nelson, D. B. Martin and M. Tewari, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 10513–10518.
- C. Alix-Panabières and K. Pantel, *Clin. Chem.*, 2013, **59**, 110–118.
- A. S. Azmi, B. Bao and F. H. Sarkar, *Cancer Metastasis Rev.*, 2013, **32**, 623–642.
- Y. M. Lo, J. Zhang, T. N. Leung, T. K. Lau, A. M. Chang and N. M. Hjelm, *Am. J. Hum. Genet.*, 1999, **64**, 218–224.
- S. P. Pricker, *Gold Bull.*, 1996, **29**, 53–60.
- J. W. Alexander, *Surg. Infect. (Larchmt)*, 2009, **10**, 289–292.
- A. J. Lewis and D. T. Walz, *Prog. Med. Chem.*, 1982, **19**, 1–58.
- S. Chernousova and M. Epple, *Angew. Chem., Int. Ed.*, 2013, **52**, 1636–1653.
- R. Mukerji, G. Mukerji and M. McGurk, *Br. J. Oral Maxillofac. Surg.*, 2006, **44**, 222–228.
- M. C. Daniel and D. Astruc, *Chem. Rev.*, 2004, **104**, 293–346.
- M. Brust and C. J. Kiely, *Colloids Surf., A*, 2002, **202**, 175–186.
- S. Mourdikoudis, R. M. Pallares and N. T. K. Thanh, *Nanoscale*, 2018, **10**, 12871–12934.
- K. Saha, S. S. Agasti, C. Kim, X. Li and V. M. Rotello, *Chem. Rev.*, 2012, **112**, 2739–2779.
- N. Li, X. Su and Y. Lu, *Analyst*, 2015, **140**, 2916–2943.
- L. Sutarlie, S. Y. Ow and X. Su, *Biotechnol. J.*, 2017, **12**, 1500459.
- R. M. Pallares, P. Choo, L. E. Cole, C. A. Mirkin, A. Lee and T. W. Odom, *Bioconjugate Chem.*, 2019, **30**, 2032–2037.
- J. Yue, R. M. Pallares, L. E. Cole, E. E. Coughlin, C. A. Mirkin, A. Lee and T. W. Odom, *ACS Appl. Mater. Interfaces*, 2018, **10**, 21920–21926.
- H. Jans and Q. Huo, *Chem. Soc. Rev.*, 2012, **41**, 2849.
- Q. Shou, C. Guo, L. Yang, L. Jia, C. Liu and H. Liu, *J. Colloid Interface Sci.*, 2011, **363**, 481–489.
- Y.-J. Kang, J.-W. Oh, Y.-R. Kim, J. S. Kim and H. Kim, *Chem. Commun.*, 2010, **46**, 5665–5667.



- 35 Y. Li, K. Burnham, J. Dykes and N. Chopra, *MRS Commun.*, 2018, **8**, 79–87.
- 36 Y. Liu, Y. Tang, N. N. Barashkov, I. S. Irgibaeva, J. W. Y. Lam, R. Hu, D. Birimzhanova, Y. Yu and B. Z. Tang, *J. Am. Chem. Soc.*, 2010, **132**, 13951–13953.
- 37 Y. Uludag and I. E. Tothill, *Anal. Chem.*, 2012, **84**, 5898–5904.
- 38 D. P. Bartel, *Cell*, 2004, **116**, 281–297.
- 39 M. B. Freidin, D. V. Freydina, M. Leung, A. M. Fernandez, A. G. Nicholson and E. Lim, *Clin. Chem.*, 2015, **61**, 1299–1304.
- 40 D. L. Peters and P. J. Pretorius, *Clin. Chim. Acta*, 2011, **412**, 806–811.
- 41 V. Swarup and M. R. Rajeswari, *FEBS Lett.*, 2007, **581**, 795–799.
- 42 E. Crowley, F. Di Nicolantonio, F. Loupakis and A. Bardelli, *Nat. Rev. Clin. Oncol.*, 2013, **10**, 472–484.
- 43 M. Yu, S. Stott, M. Toner, S. Maheswaran and D. A. Haber, *J. Cell Biol.*, 2011, **192**, 373–382.
- 44 J. Jen, L. Wu and D. Sidransky, *Ann. N. Y. Acad. Sci.*, 2010, **906**, 8–12.
- 45 J. Ko, E. Carpenter and D. Issadore, *Analyst*, 2016, **141**, 450–460.
- 46 E. Zeringer, *World J. Methodol.*, 2013, **3**, 11.
- 47 X. Huang, R. O'Connor and E. A. Kwizera, *Nanotheranostics*, 2017, **1**, 80–102.
- 48 H. J. Yoon, M. Kozminsky and S. Nagrath, *ACS Nano*, 2014, **8**, 1995–2017.
- 49 V. Myroshnychenko, J. Rodríguez-Fernández, I. Pastoriza-Santos, A. M. Funston, C. Novo, P. Mulvaney, L. M. Liz-Marzán and F. J. García de Abajo, *Chem. Soc. Rev.*, 2008, **37**, 1792–1805.
- 50 P. K. Jain, X. Huang, I. H. El-Sayed and M. A. El-Sayed, *Acc. Chem. Res.*, 2008, **41**, 1578–1586.
- 51 G. Mie, *Ann. Phys.*, 1908, **330**, 377–445.
- 52 L. M. Liz-Marzán, *Langmuir*, 2006, **22**, 32–41.
- 53 J. Pérez-Juste, I. Pastoriza-Santos, L. M. Liz-Marzán and P. Mulvaney, *Coord. Chem. Rev.*, 2005, **249**, 1870–1901.
- 54 P. K. Jain, K. S. Lee, I. H. El-Sayed and M. A. El-Sayed, *J. Phys. Chem. B*, 2006, **110**, 7238–7248.
- 55 H. Chen, X. Kou, Z. Yang, W. Ni and J. Wang, *Langmuir*, 2008, **24**, 5233–5237.
- 56 X. Liu, M. Atwater, J. Wang and Q. Huo, *Colloids Surf., B*, 2007, **58**, 3–7.
- 57 S. Link and M. El-Sayed, *J. Phys. Chem. B*, 1999, **103**, 4212–4217.
- 58 N. J. Halas, S. Lal, W.-S. Chang, S. Link and P. Nordlander, *Chem. Rev.*, 2011, **111**, 3913–3961.
- 59 K. H. Su, Q. H. Wei, X. Zhang, J. J. Mock, D. R. Smith and S. Schultz, *Nano Lett.*, 2003, **3**, 1087–1090.
- 60 L. A. Austin, B. Kang and M. A. El-Sayed, *Nano Today*, 2015, **10**, 542–558.
- 61 M. L. Juan, M. Righini and R. Quidant, *Nat. Photonics*, 2011, **5**, 349–356.
- 62 T. Chung, S. Y. Lee, E. Y. Song, H. Chun and B. Lee, *Sensors*, 2011, **11**, 10907–10929.
- 63 N. N. M. Adnan, Y. Y. Cheng, N. M. N. Ong, T. T. Kamaruddin, E. Rozlan, T. W. Schmidt, H. T. T. Duong and C. Boyer, *Polym. Chem.*, 2016, **7**, 2888–2903.
- 64 R. A. Alvarez-Puebla and L. M. Liz-Marzán, *Small*, 2010, **6**, 604–610.
- 65 C. C. Huang, Z. Yang, K. H. Lee and H. T. Chang, *Angew. Chem., Int. Ed.*, 2007, **46**, 6824–6828.
- 66 S. A. Maier, *Plasmonics: Fundamentals and applications*, Springer Science & Business Media, 2007.
- 67 P. K. Jain and M. A. El-Sayed, *Chem. Phys. Lett.*, 2010, **487**, 153–164.
- 68 G. C. Schatz, M. A. Young and R. P. Duyne, in *Electromagnetic Mechanism of SERS BT – Surface-Enhanced Raman Scattering: Physics and Applications*, ed. K. Kneipp, M. Moskovits and H. Kneipp, Springer Berlin Heidelberg, Berlin, Heidelberg, 2006, pp. 19–45.
- 69 J. A. Creighton and D. G. Eadon, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 3881–3891.
- 70 P. Kanninen, C. Johans, J. Merta and K. Kontturi, *J. Colloid Interface Sci.*, 2008, **318**, 88–95.
- 71 J. Turkevich, P. C. Stevenson and J. Hillier, *Discuss. Faraday Soc.*, 1951, **11**, 55–75.
- 72 G. Frens, *Nat. Phys. Sci.*, 1973, **241**, 20–22.
- 73 J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot and A. Plech, *J. Phys. Chem. B*, 2006, **110**, 15700–15707.
- 74 J. Polte, T. T. Ahner, F. Delissen, S. Sokolov, F. Emmerling, A. F. Thünemann and R. Kraehnert, *J. Am. Chem. Soc.*, 2010, **132**, 1296–1301.
- 75 M. Wuthschick, A. Birnbaum, S. Witte, M. Sztucki, U. Vainio, N. Pinna, K. Rademann, F. Emmerling, R. Kraehnert and J. Polte, *ACS Nano*, 2015, **9**, 7052–7071.
- 76 M. O. Besenhard, R. Baber, A. P. LaGrow, L. Mazzei, N. T. K. Thanh and A. Gavriilidis, *CrystEngComm*, 2018, **20**, 7082–7093.
- 77 M. Brust, M. Walker, D. Bethell, D. J. Schiffrin and R. Whyman, *J. Chem. Soc., Chem. Commun.*, 1994, 801.
- 78 H. Huang, H. du Toit, S. Ben Jaber, G. Wu, L. Panariello, N. T. K. Thanh, I. P. Parkin and A. Gavriilidis, *React. Chem. Eng.*, 2019, **4**, 884–890.
- 79 R. Baber, L. Mazzei, N. T. K. Thanh and A. Gavriilidis, *Nanoscale*, 2017, **9**, 14149–14161.
- 80 N. R. Jana, L. Gearheart and C. J. Murphy, *Adv. Mater.*, 2001, **13**, 1389–1393.
- 81 B. Nikoobakht and M. A. El-Sayed, *Chem. Mater.*, 2003, **15**, 1957–1962.
- 82 X. Ye, L. Jin, H. Caglayan, J. Chen, G. Xing, C. Zheng, V. Doan-Nguyen, Y. Kang, N. Engheta, C. R. Kagan and C. B. Murray, *ACS Nano*, 2012, **6**, 2804–2817.
- 83 R. M. Pallares, X. Su, S. H. Lim and N. T. K. Thanh, *J. Mater. Chem. C*, 2016, **4**, 53–61.
- 84 R. M. Pallares, Y. Wang, S. H. Lim, N. T. K. Thanh and X. Su, *Nanomedicine*, 2016, **11**, 2845–2860.
- 85 C. L. Nehl, H. Liao and J. H. Hafner, *Nano Lett.*, 2006, **6**, 683–688.



- 86 P. Senthil Kumar, I. Pastoriza-Santos, B. Rodríguez-González, F. Javier, G. de Abajo and L. M. Liz-Marzán, *Nanotechnology*, 2008, **19**, 15606.
- 87 R. M. Pallares, T. Stilson, P. Choo, J. Hu and T. W. Odom, *ACS Appl. Nano Mater.*, 2019, **2**, 5266–5271.
- 88 F. Hao, C. L. Nehl, J. H. Hafner and P. Nordlander, *Nano Lett.*, 2007, **7**, 729–732.
- 89 L. Rodríguez-Lorenzo, R. A. Álvarez-Puebla, I. Pastoriza-Santos, S. Mazzucco, O. Stéphan, M. Kociak, L. M. Liz-Marzán and F. J. G. De Abajo, *J. Am. Chem. Soc.*, 2009, **131**, 4616–4618.
- 90 P. C. Lee and D. Meisel, *J. Phys. Chem.*, 1982, **86**, 3391–3395.
- 91 P. V. Kamat, M. Flumiani and G. V. Hartland, *J. Phys. Chem. B*, 1998, **102**, 3123–3128.
- 92 R. S. Sheng, L. Zhu and M. D. Morris, *Anal. Chem.*, 1986, **58**, 1116–1119.
- 93 A. Henglein and M. Giersig, *J. Phys. Chem. B*, 1999, **103**, 9533–9539.
- 94 R. Baber, L. Mazzei, N. T. K. Thanh and A. Gavriilidis, *J. Flow Chem.*, 2016, **6**, 268–278.
- 95 K. S. Lee and M. A. El-Sayed, *J. Phys. Chem. B*, 2006, **110**, 7238–7248.
- 96 R. Jin, *Nanoscale*, 2010, **2**, 343–362.
- 97 Y. Lu and W. Chen, *Chem. Soc. Rev.*, 2012, **41**, 3594–3623.
- 98 O. Varnavski, G. Ramakrishna, J. Kim, D. Lee and T. Goodson, *J. Am. Chem. Soc.*, 2010, **132**, 16–17.
- 99 H. Häkkinen, *Nat. Chem.*, 2012, **4**, 443–455.
- 100 Y. Li, G. Galli and F. Gygi, *ACS Nano*, 2008, **2**, 1896–1902.
- 101 L. Shang, S. Dong and G. U. Nienhaus, *Nano Today*, 2011, **6**, 401–418.
- 102 Y. Yu, Q. Yao, Z. Luo, X. Yuan, J. Y. Lee and J. Xie, *Nanoscale*, 2013, **5**, 4606–4620.
- 103 S. Chen, R. S. Ingram, M. J. Hostetler, J. J. Pietron, R. W. Murray, T. G. Schaaff, J. T. Khoury, M. M. Alvarez and R. L. Whetten, *Science*, 1998, **280**, 2098–2101.
- 104 W. Chen and S. Chen, *Angew. Chem., Int. Ed.*, 2009, **48**, 4386–4389.
- 105 Y. Liu, H. Tsunoyama, T. Akita and T. Tsukuda, *Chem. Commun.*, 2010, **46**, 550–552.
- 106 Y. Liu, H. Tsunoyama, T. Akita, S. Xie and T. Tsukuda, *ACS Catal.*, 2011, **1**, 2–6.
- 107 G. Ramakrishna, O. Varnavski, J. Kim, D. Lee and T. Goodson, *J. Am. Chem. Soc.*, 2008, **130**, 5032–5033.
- 108 M. A. Habeeb Muhammed, A. K. Shaw, S. K. Pal and T. Pradeep, *J. Phys. Chem. C*, 2008, **112**, 14324–14330.
- 109 Z. Wu and R. Jin, *Nano Lett.*, 2010, **10**, 2568–2573.
- 110 S. Link, A. Beeby, S. FitzGerald, M. A. El-Sayed, T. G. Schaaff and R. L. Whetten, *J. Phys. Chem. B*, 2002, **106**, 3410–3415.
- 111 Y. Negishi, Y. Takasugi, S. Sato, H. Yao, K. Kimura and T. Tsukuda, *J. Am. Chem. Soc.*, 2004, **126**, 6518–6519.
- 112 Y. Negishi, K. Nobusada and T. Tsukuda, *J. Am. Chem. Soc.*, 2005, **127**, 5261–5270.
- 113 J. T. Petty, J. Zheng, N. V. Hud and R. M. Dickson, *J. Am. Chem. Soc.*, 2004, **126**, 5207–5212.
- 114 H. C. Yeh, J. Sharma, J. J. Han, J. S. Martinez and J. H. Werner, *Nano Lett.*, 2010, **10**, 3106–3110.
- 115 L. Sutarlie, K. M. M. Aung, M. G. L. Lim, S. Lukman, E. Cheung and X. Su, *Plasmonics*, 2014, **9**, 753–763.
- 116 J. Sun, Y. Xianyu and X. Jiang, *Chem. Soc. Rev.*, 2014, **43**, 6239–6253.
- 117 H. Nishi, K. Asami and T. Tatsuma, *Opt. Mater. Express*, 2016, **6**, 1043.
- 118 S. K. Ghosh and T. Pal, *Chem. Rev.*, 2007, **107**, 4797–4862.
- 119 R. Liu, R. Liew, J. Zhou and B. Xing, *Angew. Chem.*, 2007, **119**, 8955–8959.
- 120 N. Nath and A. Chilkoti, *Anal. Chem.*, 2002, **74**, 504–509.
- 121 J. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2003, **125**, 6642–6643.
- 122 C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607–609.
- 123 T. M. H. Lee, *Sensors*, 2008, **8**, 5535–5559.
- 124 L. R. Hirsch, J. B. Jackson, A. Lee, N. J. Halas and J. L. West, *Anal. Chem.*, 2003, **75**, 2377–2381.
- 125 N. T. Kim Thanh and Z. Rosenzweig, *Anal. Chem.*, 2002, **74**, 1624–1628.
- 126 R. M. Pallares, M. Bosman, N. T. K. Thanh and X. Su, *Nanoscale*, 2016, **8**, 19973–19977.
- 127 R. M. Pallares, S. L. Kong, T. H. Ru, N. T. K. Thanh, Y. Lu and X. Su, *Chem. Commun.*, 2015, **51**, 14524–14527.
- 128 R. Kanjanawarut and X. Su, *Anal. Chem.*, 2009, **81**, 6122–6129.
- 129 X. Su and R. Kanjanawarut, *ACS Nano*, 2009, **3**, 2751–2759.
- 130 A. P. Demchenko, *Methods Appl. Fluoresc.*, 2013, **1**.
- 131 R. M. Pallares, L. Sutarlie, N. T. K. Thanh and X. Su, *Sens. Actuators, B*, 2018, **271**, 97–103.
- 132 L. Dykman and N. Khlebtsov, *Chem. Soc. Rev.*, 2012, **41**, 2256–2282.
- 133 K. A. Willets and R. P. Van Duyne, *Annu. Rev. Phys. Chem.*, 2007, **58**, 267–297.
- 134 J. G. Gordon and S. Ernst, *Surf. Sci.*, 1980, **101**, 499–506.
- 135 C. Nylander, B. Liedberg and T. Lind, *Sens. Actuators*, 1982, **3**, 79–88.
- 136 J. Homola, *Chem. Rev.*, 2008, **108**, 462–493.
- 137 J. Ye, K. Bonroy, D. Nelis, F. Frederix, J. D'Haen, G. Maes and G. Borghs, *Colloids Surf., A*, 2008, **321**, 313–317.
- 138 N. Verellen, P. Van Dorpe, C. Huang, K. Lodewijks, G. A. E. Vandenbosch, L. Lagae and V. V. Moshchalkov, *Nano Lett.*, 2011, **11**, 391–397.
- 139 A. G. Brolo, R. Gordon, B. Leathem and K. L. Kavanagh, *Langmuir*, 2004, **20**, 4813–4815.
- 140 A. V. Whitney, J. W. Elam, S. Zou, A. V. Zinovev, P. C. Stair, G. C. Schatz and R. P. Van Duyne, *J. Phys. Chem. B*, 2005, **109**, 20522–20528.
- 141 E. Smith and G. Dent, *Modern Raman Spectroscopy – A Practical Approach*, John Wiley & Sons, New York, 2005.
- 142 K. Kneipp, H. Kneipp, I. Itzkan, R. R. Dasari and M. S. Feld, *Chem. Rev.*, 1999, **99**, 2957–2976.
- 143 M. J. Banholzer, J. E. Millstone, L. Qin and C. A. Mirkin, *Chem. Soc. Rev.*, 2008, **37**, 885–897.
- 144 K. Kneipp, H. Kneipp and J. Kneipp, *Acc. Chem. Res.*, 2006, **39**, 443–450.





- 145 W. E. Doering and S. Nie, *J. Phys. Chem. B*, 2002, **106**, 311–317.
- 146 P. G. Etchegoin and E. C. Le Ru, *Phys. Chem. Chem. Phys.*, 2008, **10**, 6079–6089.
- 147 H. Xu, E. Bjerneld, M. Käll and L. Börjesson, *Phys. Rev. Lett.*, 1999, **83**, 4357–4360.
- 148 B. N. J. Persson, *Chem. Phys. Lett.*, 1981, **82**, 561–565.
- 149 A. Otto, *J. Raman Spectrosc.*, 2005, **36**, 497–509.
- 150 X. X. Han, B. Zhao and Y. Ozaki, *Anal. Bioanal. Chem.*, 2009, **394**, 1719–1727.
- 151 R. Thompson, in *Fluorescence sensors and biosensors*, CRC press, Boca Raton, FL, 2005, p. 377.
- 152 K. Aslan, I. Gryczynski, J. Malicka, E. Matveeva, J. R. Lakowicz and C. D. Geddes, *Curr. Opin. Biotechnol.*, 2005, **16**, 55–62.
- 153 X. Kong, E. Nir, K. Hamadani and S. Weiss, *J. Am. Chem. Soc.*, 2007, **129**, 4643–4654.
- 154 V. L. Mosiman, B. K. Patterson, L. Canterero and C. L. Goolsby, *Commun. Clin. Cytom.*, 1997, **30**, 151–156.
- 155 E. Dulkeith, A. C. Morteani, T. Niedereichholz, T. A. Klar, J. Feldmann, S. A. Levi, F. C. J. M. van Veggel, D. N. Reinhoudt, M. Möller and D. I. Gittins, *Phys. Rev. Lett.*, 2002, **89**, 203002.
- 156 J. Gersten and A. Nitzan, *J. Chem. Phys.*, 1981, **75**, 1139–1152.
- 157 E. A. Jares-Erijman and T. M. Jovin, *Nat. Biotechnol.*, 2003, **21**, 1387–1395.
- 158 T. L. Jennings, M. P. Singh and G. F. Strouse, *J. Am. Chem. Soc.*, 2006, **128**, 5462–5467.
- 159 C. C. Huang and H. T. Chang, *Anal. Chem.*, 2006, **78**, 8332–8338.
- 160 B.-C. Ye and B.-C. Yin, *Angew. Chem., Int. Ed.*, 2008, **47**, 8386–8389.
- 161 E. Oh, M.-Y. Hong, D. Lee, S.-H. Nam, H. C. Yoon and H.-S. Kim, *J. Am. Chem. Soc.*, 2005, **127**, 3270–3271.
- 162 Y. P. Kim, Y. H. Oh, E. Oh, S. Ko, M. K. Han and H. S. Kim, *Anal. Chem.*, 2008, **80**, 4634–4641.
- 163 M. Li, Q. Wang, X. Shi, L. A. Hornak and N. Wu, *Anal. Chem.*, 2011, **83**, 7061–7065.
- 164 J. M. Liu, J. T. Chen and X. P. Yan, *Anal. Chem.*, 2013, **85**, 3238–3245.
- 165 J.-L. Ma, B.-C. Yin, H.-N. Le and B.-C. Ye, *ACS Appl. Mater. Interfaces*, 2015, **7**, 12856–12863.
- 166 S. Lukman, K. M. M. Aung, J. Liu, B. Liu and X. Su, *ACS Appl. Mater. Interfaces*, 2013, **5**, 12725–12734.
- 167 S. Lukman, K. M. Moh Aung, M. G. Liang Lim, S. Hong, S. K. Tan, E. Cheung and X. Su, *RSC Adv.*, 2014, **4**, 8883.
- 168 X. He, H. Liu, Y. Li, S. Wang, Y. Li, N. Wang, J. Xiao, X. Xu and D. Zhu, *Adv. Mater.*, 2005, **17**, 2811–2815.
- 169 N. Zhang, Y. Liu, L. Tong, K. Xu, L. Zhuo and B. Tang, *Analyst*, 2008, **133**, 1176–1181.
- 170 J.-H. Lin, C.-W. Chang and W.-L. Tseng, *Analyst*, 2010, **135**, 104–110.
- 171 M. De, S. Rana, H. Akpınar, O. R. Miranda, R. R. Arvizo, U. H. F. Bunz and V. M. Rotello, *Nat. Chem.*, 2009, **1**, 461–465.
- 172 R. L. Phillips, O. R. Miranda, C.-C. You, V. M. Rotello and U. H. F. Bunz, *Angew. Chem., Int. Ed.*, 2008, **47**, 2590–2594.
- 173 B. Dubertret, M. Calame and A. J. Libchaber, *Nat. Biotechnol.*, 2001, **19**, 365–370.
- 174 P. C. Ray, A. Fortner and G. K. Darbha, *J. Phys. Chem. B*, 2006, **110**, 20745–20748.
- 175 R. Bardhan, N. K. Grady, J. R. Cole, A. Joshi and N. J. Halas, *ACS Nano*, 2009, **3**, 744–752.
- 176 Y. Fu, J. Zhang and J. R. Lakowicz, *J. Am. Chem. Soc.*, 2010, **132**, 5540–5541.
- 177 D. Nepal, L. F. Drummy, S. Biswas, K. Park and R. A. Vaia, *ACS Nano*, 2013, **7**, 9064–9074.
- 178 N. S. Abadeer, M. R. Brennan, W. L. Wilson and C. J. Murphy, *ACS Nano*, 2014, **8**, 8392–8406.
- 179 N. Gandra, C. Portz, L. Tian, R. Tang, B. Xu, S. Achilefu and S. Singamaneni, *Angew. Chem., Int. Ed.*, 2014, **53**, 866–870.
- 180 C. D. Geddes and J. R. Lakowicz, *J. Fluoresc.*, 2002, **12**, 121–129.
- 181 K. Aslan, S. N. Malyn and C. D. Geddes, *J. Fluoresc.*, 2007, **17**, 7–13.
- 182 F. Xie, M. S. Baker and E. M. Goldys, *Chem. Mater.*, 2008, **20**, 1788–1797.
- 183 Y. Cheng, T. Stakenborg, P. Van Dorpe, L. Lagae, M. Wang, H. Chen and G. Borghs, *Anal. Chem.*, 2011, **83**, 1307–1314.
- 184 S. H. Lee, S. M. Wabaidur, Z. A. Alothman and S. M. Alam, *Luminescence*, 2011, **26**, 768–773.
- 185 S. Guo and E. Wang, *Anal. Chim. Acta*, 2007, **598**, 181–192.
- 186 J. M. Pingarrón, P. Yáñez-Sedeño and A. González-Cortés, *Electrochim. Acta*, 2008, **53**, 5848–5866.
- 187 J. Wang, *Analyst*, 2005, **130**, 421–426.
- 188 X. Luo, A. Morrin, A. J. Killard and M. R. Smyth, *Electroanalysis*, 2006, **18**, 319–326.
- 189 P. M. P. Mandel, *C. R. Acad. Sci.*, 1948, **142**, 241–243.
- 190 N. Suzuki, A. Kamataki, J. Yamaki and Y. Homma, *Clin. Chim. Acta*, 2008, **387**, 55–58.
- 191 K. Jung, M. Fleischhacker and A. Rabien, *Clin. Chim. Acta*, 2010, **411**, 1611–1624.
- 192 Y. Li, A. W. Wark, H. J. Lee and R. M. Corn, *Anal. Chem.*, 2006, **78**, 3158–3164.
- 193 S. Jahr, H. Hentze, S. Englisch, D. Hardt, F. O. Fackelmayer, R. D. Hesch and R. Knippers, *Cancer Res.*, 2001, **61**, 1659–1665.
- 194 H. Schwarzenbach, D. S. B. Hoon and K. Pantel, *Nat. Rev. Cancer*, 2011, **11**, 426–437.
- 195 C. Bettgowda, M. Sausen, R. J. Leary, I. Kinde, Y. Wang, N. Agrawal, B. R. Bartlett, H. Wang, B. Lubber, R. M. Alani, *et al.*, *Sci. Transl. Med.*, 2014, **6**, 224ra24–224ra24.
- 196 S. J. Dawson, D. W. Tsui, M. Murtaza, H. Biggs, O. M. Rueda, S. F. Chin, M. J. Dunning, D. Gale, T. Forshew, B. Mahler-Araujo and S. Rajan, *N. Engl. J. Med.*, 2013, **368**, 1199–1209.
- 197 R. van Eijk, J. Licht, M. Schruppf, M. T. Yazdi, D. Ruano, G. I. Forte, P. M. Nederlof, M. Veselic, K. F. Rabe,



- J. T. Annema, V. Smit, H. Morreau and T. van Wezel, *PLoS One*, 2011, **6**, e17791.
- 198 A. Strati, A. Markou, C. Parisi, E. Politaki, D. Mavroudis, V. Georgoulas and E. Lianidou, *BMC Cancer*, 2011, **11**, 422.
- 199 R. J. Leary, I. Kinde, F. Diehl, K. Schmidt, C. Clouser, C. Duncan, A. Antipova, C. Lee, K. McKernan, F. M. De La Vega, K. W. Kinzler, B. Vogelstein, L. A. Diaz and V. E. Velculescu, *Sci. Transl. Med.*, 2010, **2**, 20ra14–20ra14.
- 200 R. J. Leary, J. Cummins, T. L. Wang and V. E. Velculescu, *Nat. Protoc.*, 2007, **2**, 1973.
- 201 H. Ito, H. Inoue, K. Hasegawa, Y. Hasegawa, T. Shimizu, S. Kimura, M. Onimaru, H. Ikeda and S. ei Kudo, *Nanomedicine*, 2014, **10**, 599–608.
- 202 H. Ito, K. Hasegawa, Y. Hasegawa, T. Nishimaki, K. Hosomichi, S. Kimura, M. Ohba, H. Yao, M. Onimaru, I. Inoue and H. Inoue, *Sci. Rep.*, 2015, **5**, 10455.
- 203 R. M. Pallares, N. T. K. Thanh and X. Su, *Chem. Commun.*, 2018, **54**, 11260–11263.
- 204 R. Sachidanandam, D. Weissman, S. C. Schmidt, J. M. Kakol, L. D. Stein, G. Marth, S. Sherry, J. C. Mullikin, B. J. Mortimore, D. L. Willey, S. E. Hunt, C. G. Cole, P. C. Coggill, C. M. Rice, Z. Ning, J. Rogers, D. R. Bentley, P. Y. Kwok, E. R. Mardis, R. T. Yeh, B. Schultz, L. Cook, R. Davenport, M. Dante, L. Fulton, L. Hillier, R. H. Waterston, J. D. McPherson, B. Gilman, S. Schaffner, W. J. Van Etten, D. Reich, J. Higgins, M. J. Daly, B. Blumenstiel, J. Baldwin, N. Stange-Thomann, M. C. Zody, L. Linton, E. S. Lander and D. Altshuler, *Nature*, 2001, **409**, 928.
- 205 E. R. Bleecker, W. B. Isaacs, A. R. Turner, H. Grönberg, D. Duggan, Y. Zhu, S. Smith, P. Stattin, S. L. Zheng, J. Sun, G. Li, F.-C. Hsu, D. A. Meyers, J. D. Carpten, B.-L. Chang, A. K. Kader, F. Wiklund, W. Liu, H.-O. Adami, K. Bälter and J. Xu, *N. Engl. J. Med.*, 2008, **358**, 910–919.
- 206 H. Lee, S. W. Joo, S. Y. Lee, C. H. Lee, K. A. Yoon and K. Lee, *Biosens. Bioelectron.*, 2010, **26**, 730–735.
- 207 H. Lee, T. Kang, K. A. Yoon, S. Y. Lee, S. W. Joo and K. Lee, *Biosens. Bioelectron.*, 2010, **25**, 1669–1674.
- 208 H. Li and L. J. Rothberg, *J. Am. Chem. Soc.*, 2004, **126**, 10958–10961.
- 209 Y. L. Jung, C. Jung, H. Parab, D. Y. Cho and H. G. Park, *ChemBioChem*, 2011, **12**, 1387–1390.
- 210 J. Li, X. Chu, Y. Liu, J. H. Jiang, Z. He, Z. Zhang, G. Shen and R. Q. Yu, *Nucleic Acids Res.*, 2005, **33**, 1–9.
- 211 Y. S. Huh, A. J. Lowe, A. D. Strickland, C. A. Batt and D. Erickson, *J. Am. Chem. Soc.*, 2009, **131**, 2208–2213.
- 212 A. J. Lowe, Y. S. Huh, A. D. Strickland, D. Erickson and C. A. Batt, *Anal. Chem.*, 2010, **82**, 5810–5814.
- 213 W. Shen, H. Deng, A. K. L. Teo and Z. Gao, *Chem. Commun.*, 2012, **48**, 10225–10227.
- 214 Q. Wang, L. Yang, X. Yang, K. Wang, L. He and J. Zhu, *Anal. Chim. Acta*, 2011, **688**, 163–167.
- 215 P. Valentini, R. Fiammengo, S. Sabella, M. Gariboldi, G. Maiorano, R. Cingolani and P. P. Pompa, *ACS Nano*, 2013, **7**, 5530–5538.
- 216 S. Q. Huang, J. Hu, G. Zhu and C. Y. Zhang, *Biosens. Bioelectron.*, 2015, **65**, 191–197.
- 217 D. Kato and M. Oishi, *ACS Nano*, 2014, **8**, 9988–9997.
- 218 S. S. Jeffrey, A.-L. Borresen-Dale, C. A. Rees, R. Tibshirani, P. E. Lonning, J. R. Pollack, P. O. Brown, T. Sorlie, D. Botstein and C. M. Perou, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 12963–12968.
- 219 J. R. Pollack, C. M. Perou, A. A. Alizadeh, M. B. Eisen, A. Pergamenschikov, C. F. Williams, S. S. Jeffrey, D. Botstein and P. O. Brown, *Nat. Genet.*, 1999, **23**, 41–46.
- 220 T. A. Taton, C. A. Mirkin and R. L. Letsinger, *Science*, 2000, **289**, 1757–1760.
- 221 J. J. Storhoff, S. S. Marla, P. Bao, S. Hagenow, H. Mehta, A. Lucas, V. Garimella, T. Patno, W. Buckingham, W. Cork and U. R. Müller, *Biosens. Bioelectron.*, 2004, **19**, 875–883.
- 222 Y. P. Bao, M. Huber, T. F. Wei, S. S. Marla, J. J. Storhoff and U. R. Müller, *Nucleic Acids Res.*, 2005, **33**, e15.
- 223 T. H. Rabbitts and T. H. Rabbitts, *Nature*, 1994, **372**, 143–149.
- 224 N. Pallisgaard, P. Hokland, D. C. Riishøj, B. Pedersen and P. Jørgensen, *Blood*, 1998, **92**, 574–588.
- 225 K. Edwards, C. Johnstone and C. Thompson, *Nucleic Acids Res.*, 1991, **19**, 1349.
- 226 D. P. Kalogianni, V. Bravou, T. K. Christopoulos, P. C. Ioannou and N. C. Zoumbos, *Nucleic Acids Res.*, 2007, **35**, e23.
- 227 M. Li, W. D. Chen, N. Papadopoulos, S. N. Goodman, N. C. Bjerregaard, S. Laurberg, B. Levin, H. Juhl, N. Arber, H. Moinova, K. Durkee, K. Schmidt, Y. He, F. Diehl, V. E. Velculescu, S. Zhou, L. A. Diaz, K. W. Kinzler, S. D. Markowitz and B. Vogelstein, *Nat. Biotechnol.*, 2009, **27**, 858–863.
- 228 A. H. Nguyen and S. J. Sim, *Biosens. Bioelectron.*, 2014, **67**, 443–449.
- 229 J. Hu and C.-y Zhang, *Biosens. Bioelectron.*, 2012, **31**, 451–457.
- 230 H. Zhang, M. Li, M. Fan, J. Gu, P. Wu and C. Cai, *Chem. Commun.*, 2014, **50**, 2932–2934.
- 231 Filled.
- 232 L. He and G. J. Hannon, *Nat. Rev. Genet.*, 2004, **5**, 522.
- 233 H. Dong, J. Lei, L. Ding, Y. Wen, H. Ju and X. Zhang, *Chem. Rev.*, 2013, **113**, 6207–6233.
- 234 J. Brennecke, D. R. Hipfner, A. Stark, R. B. Russell and S. M. Cohen, *Cell*, 2003, **113**, 25–36.
- 235 P. Xu, S. Y. Vernooy, M. Guo and B. A. Hay, *Curr. Biol.*, 2003, **13**, 790–795.
- 236 V. Ambros, *Cell*, 2003, **113**, 673–676.
- 237 C. Z. Chen, L. Li, H. F. Lodish and D. P. Bartel, *Science*, 2004, **5654**, 83–86.
- 238 W. C. S. Cho, *Biochim. Biophys. Acta, Rev. Cancer*, 2010, **1805**, 209–217.
- 239 T. Dalmay and D. R. Edwards, *Oncogene*, 2006, **25**, 6170.
- 240 J. Kocerha, S. Kauppinen and C. Wahlestedt, *Neuromol. Med.*, 2009, **11**, 162–172.
- 241 A. K. Pandey, P. Agarwal, K. Kaur and M. Datta, *Cell. Physiol. Biochem.*, 2009, **23**, 221–232.



- 242 S. Saal and S. J. Harvey, *Curr. Opin. Nephrol. Hypertens.*, 2009, **18**, 317–323.
- 243 X. M. Chen, *World J. Gastroenterol.*, 2009, **15**, 1665.
- 244 C. López-Camarillo and L. A. Marchat, *MicroRNAs in cancer*, CRC Press, Boca Raton, FL, 2013.
- 245 G. Di Leva and C. M. Croce, *Trends Mol. Med.*, 2010, **16**, 257–267.
- 246 C. Love and S. Dave, in *Hematological Malignancies*, Humana Press, Totowa, NJ, 2013, pp. 285–296.
- 247 P. S. Mitchell, R. K. Parkin, E. M. Kroh, B. R. Fritz, S. K. Wyman, E. L. Pogossova-Agadjanyan, A. Peterson, J. Noteboom, K. C. O'Briant, A. Allen, D. W. Lin, N. Urban, C. W. Drescher, B. S. Knudsen, D. L. Stirewalt, R. Gentleman, R. L. Vessella, P. S. Nelson, D. B. Martin and M. Tewari, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 10513–10518.
- 248 H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee and J. O. Lötvall, *Nat. Cell Biol.*, 2007, **9**, 654.
- 249 J. D. Arroyo, J. R. Chevillet, E. M. Kroh, I. K. Ruf, C. C. Pritchard, D. F. Gibson, P. S. Mitchell, C. F. Bennett, E. L. Pogossova-Agadjanyan, D. L. Stirewalt, J. F. Tait and M. Tewari, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 5003–5008.
- 250 M. Accerbi, S. A. Schmidt, E. De Paoli, S. Park, D.-H. Jeong and P. J. Green, in *Methods for Isolation of Total RNA to Recover miRNAs and Other Small RNAs from Diverse Species BT – Plant MicroRNAs: Methods and Protocols*, ed. B. C. Meyers and P. J. Green, Humana Press, Totowa, NJ, 2010, pp. 31–50.
- 251 K. A. Cissell, S. Shrestha and S. K. Deo, *Anal. Chem.*, 2007, 4754–4761.
- 252 Y. Tu, P. Wu, H. Zhang and C. Cai, *Chem. Commun.*, 2012, **48**, 10718–10720.
- 253 S. W. Yang and T. Vosch, *Anal. Chem.*, 2011, **83**, 6935–6939.
- 254 F. Degliangeli, P. Kshirsagar, V. Brunetti, P. P. Pompa and R. Fiammengo, *J. Am. Chem. Soc.*, 2014, **136**, 2264–2267.
- 255 Y. Q. Liu, M. Zhang, B. C. Yin and B. C. Ye, *Anal. Chem.*, 2012, **84**, 5165–5169.
- 256 H. Dong, S. Jin, H. Ju, K. Hao, L. P. Xu, H. Lu and X. Zhang, *Anal. Chem.*, 2012, **84**, 8670–8674.
- 257 H. Yin, Y. Zhou, C. Chen, L. Zhu and S. Ai, *Analyst*, 2012, **137**, 1389–1395.
- 258 H. Yin, Y. Zhou, H. Zhang, X. Meng and S. Ai, *Biosens. Bioelectron.*, 2012, **33**, 247–253.
- 259 N. Xia, L. Zhang, G. Wang, Q. Feng and L. Liu, *Biosens. Bioelectron.*, 2013, **47**, 461–466.
- 260 J. Wang, X. Yi, H. Tang, H. Han, M. Wu and F. Zhou, *Anal. Chem.*, 2012, **84**, 6400–6406.
- 261 J. Mandli, H. Mohammadi and A. Amine, *Bioelectrochemistry*, 2017, **116**, 17–23.
- 262 H. Zhang, M. Fan, J. Jiang, Q. Shen, C. Cai and J. Shen, *Anal. Chim. Acta*, 2019, **1064**, 33–39.
- 263 J. D. Driskell, A. G. Seto, L. P. Jones, S. Jokela, R. A. Dluhy, Y. P. Zhao and R. A. Tripp, *Biosens. Bioelectron.*, 2008, **24**, 917–922.
- 264 Y. Chen, G. Chen, S. Feng, J. Pan, X. Zheng, Y. Su, Y. Chen, Z. Huang, X. Lin, F. Lan, R. Chen and H. Zeng, *J. Biomed. Opt.*, 2012, **17**, 67003.
- 265 R. D. Li, B. C. Yin and B. C. Ye, *Biosens. Bioelectron.*, 2016, **86**, 1011–1016.
- 266 P. Miao, Y. Tang, Z. Mao and Y. Liu, *Part. Part. Syst. Charact.*, 2017, **34**, 1600405.
- 267 W. J. Yang, X. B. Li, Y. Y. Li, L. F. Zhao, W. L. He, Y. Q. Gao, Y. J. Wan, W. Xia, T. Chen, H. Zheng, M. Li and S.-q. Xu, *Anal. Biochem.*, 2008, **376**, 183–188.
- 268 Y.-S. Borghei, M. Hosseini and M. R. Ganjali, *Clin. Chim. Acta*, 2018, **483**, 119–125.
- 269 S. Fang, H. J. Lee, A. W. Wark and R. M. Corn, *J. Am. Chem. Soc.*, 2006, **128**, 14044–14046.
- 270 M. Castoldi, S. Schmidt, V. Benes, M. Noerholm, A. E. Kulozik, M. W. Hentze and M. U. Muckenthaler, *RNA*, 2006, **12**, 913–920.
- 271 A. H. Alhasan, D. Y. Kim, W. L. Daniel, E. Watson, J. J. Meeks, C. S. Thaxton and C. A. Mirkin, *Anal. Chem.*, 2012, **84**, 4153–4160.
- 272 M. Cristofanilli, G. T. Budd, M. J. Ellis, A. Stopeck, J. Matera, M. C. Miller, J. M. Reuben, G. V. Doyle, W. J. Allard, L. W. M. M. Terstappen and D. F. Hayes, *N. Engl. J. Med.*, 2004, **351**, 781–791.
- 273 S. J. Cohen, C. J. A. Punt, N. Iannotti, B. H. Saidman, K. D. Sabbath, N. Y. Gabrail, J. Picus, M. Morse, E. Mitchell, M. C. Miller, G. V. Doyle, H. Tissing, L. W. M. M. Terstappen and N. J. Meropol, *J. Clin. Oncol.*, 2008, **26**, 3213–3221.
- 274 J. S. de Bono, H. I. Scher, R. B. Montgomery, C. Parker, M. C. Miller, H. Tissing, G. V. Doyle, L. W. W. M. Terstappen, K. J. Pienta and D. Raghavan, *Clin. Cancer Res.*, 2008, **14**, 6302–6309.
- 275 I. Baccelli, A. Schneeweiss, S. Riethdorf, A. Stenzinger, A. Schillert, V. Vogel, C. Klein, M. Saini, T. Bauerle, M. Wallwiener, T. Holland-Letz, T. Hofner, M. Sprick, M. Scharpf, F. Marme, H. P. Sinn, K. Pantel, W. Weichert and A. Trumpp, *Nat. Biotechnol.*, 2013, **31**, 539–544.
- 276 M. I. Khan, A. M. Czarnecka, R. Duchnowska, W. Kukwa and C. Szczylik, *Curr. Signal Transduction Ther.*, 2014, **8**, 240–246.
- 277 C. L. Chaffer and R. A. Weinberg, *Science*, 2011, **331**, 1559–1564.
- 278 A. H. Talasz, A. A. Powell, D. E. Huber, J. G. Berbee, K.-H. Roh, W. Yu, W. Xiao, M. M. Davis, R. F. Pease, M. N. Mindrinos, S. S. Jeffrey and R. W. Davis, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 3970–3975.
- 279 S. Sieben, C. Bergemann, A. Lübke, B. Brockmann and D. Rescheleit, *J. Magn. Magn. Mater.*, 2001, **225**, 175–179.
- 280 R. E. Zigeuner, R. Riesenberger, H. Pohla, A. Hofstetter and R. Oberneder, *J. Urol.*, 2003, **169**, 701–705.
- 281 S. Nagrath, L. V. Sequist, S. Maheswaran, D. W. Bell, D. Irimia, L. Ulkus, M. R. Smith, E. L. Kwak, S. Digumarthy, A. Muzikansky and P. Ryan, *Nature*, 2007, **450**, 1235–1239.



- 282 S. L. Stott, C.-H. Hsu, D. I. Tsukrov, M. Yu, D. T. Miyamoto, B. A. Waltman, S. M. Rothenberg, A. M. Shah, M. E. Smas, G. K. Korir, F. P. Floyd, A. J. Gilman, J. B. Lord, D. Winokur, S. Springer, D. Irimia, S. Nagrath, L. V. Sequist, R. J. Lee, K. J. Isselbacher, S. Maheswaran, D. A. Haber and M. Toner, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 18392–18397.
- 283 A. A. Adams, P. I. Okagbare, J. Feng, M. L. Hupert, D. Patterson, J. Götten, R. L. McCarley, D. Nikitopoulos, M. C. Murphy and S. A. Soper, *J. Am. Chem. Soc.*, 2008, **130**, 8633–8641.
- 284 S. Jeon, J. M. Moon, E. S. Lee, Y. H. Kim and Y. Cho, *Angew. Chem., Int. Ed.*, 2014, **53**, 4597–4602.
- 285 W. Y. Hong, S. H. Jeon, E. S. Lee and Y. Cho, *Biomaterials*, 2014, **35**, 9573–9580.
- 286 H. W. Hou, M. E. Warkiani, B. L. Khoo, Z. R. Li, R. A. Soo, D. S.-W. Tan, W.-T. Lim, J. Han, A. A. S. Bhagat and C. T. Lim, *Sci. Rep.*, 2013, **3**, 1259.
- 287 S. Zheng, H. Lin, J. Q. Liu, M. Balic, R. Datar, R. J. Cote and Y. C. Tai, *J. Chromatogr. A*, 2007, **1162**, 154–161.
- 288 R. A. Burrell, N. McGranahan, J. Bartek and C. Swanton, *Nature*, 2013, **501**, 338–345.
- 289 V. Almendro, A. Marusyk and K. Polyak, *Annu. Rev. Pathol.*, 2013, **8**, 277–302.
- 290 C. E. Meacham and S. J. Morrison, *Nature*, 2013, **501**, 328–337.
- 291 R. Gertler, R. Rosenberg, K. Fuehrer, M. Dahm, H. Nekarda and J. R. Siewert, *Recent Results Cancer Res.*, 2003, **162**, 149–155.
- 292 V. Müller, N. Stahmann, S. Riethdorf, T. Rau, T. Zabel, A. Goetz, F. Jänicke and K. Pantel, *Clin. Cancer Res.*, 2005, **11**, 3678–3685.
- 293 T. M. Morgan, P. H. Lange and R. L. Vessella, *Front. Biosci.*, 2007, **12**, 3000–3009.
- 294 G. Vona, A. Sabile, M. Louha, V. Sitruk, S. Romana, D. Franco, M. Pazzagli, M. Vekemans, B. Lacour and P. Paterlini-bre, *Am. J. Pathol.*, 2000, **156**, 57–63.
- 295 H. Mohamed, M. Murray, J. N. Turner and M. Caggana, *J. Chromatogr. A*, 2009, **1216**, 8289–8295.
- 296 S. J. Tan, L. Yobas, G. Y. H. Lee, C. N. Ong and C. T. Lim, *Biomed. Microdevices*, 2009, **11**, 883–892.
- 297 S. Riethdorf, H. Fritsche, V. Müller, T. Rau, C. Schindlbeck, B. Rack, W. Janni, C. Coith, K. Beck, F. Jänicke, S. Jackson, T. Gornet, M. Cristofanilli and K. Pantel, *Clin. Cancer Res.*, 2007, **13**, 920–928.
- 298 W. J. Allard, J. Matera, M. C. Miller, M. Repollet, M. C. Connelly, C. Rao, A. G. J. Tibbe, J. W. Uhr and L. W. M. M. Terstappen, *Clin. Cancer Res.*, 2004, **10**, 6897–6904.
- 299 S. Maheswaran, L. V. Sequist, S. Nagrath, L. Ulkus, B. Brannigan, C. V. Collura, E. Inserra, S. Diederichs, A. J. Iafrate, D. W. Bell, S. Digumarthy, A. Muzikansky, D. Irimia, J. Settleman, R. G. Tompkins, T. J. Lynch, M. Toner and D. A. Haber, *N. Engl. J. Med.*, 2008, **359**, 366–377.
- 300 S. M. Shamah, J. M. Healy and S. T. Cload, *Acc. Chem. Res.*, 2008, **41**, 130–138.
- 301 W. Lu, A. K. Singh, S. A. Khan, D. Senapati, H. Yu and P. C. Ray, *J. Am. Chem. Soc.*, 2010, **132**, 18103–18114.
- 302 L. Beqa, Z. Fan, A. K. Singh, D. Senapati and P. C. Ray, *ACS Appl. Mater. Interfaces*, 2011, **3**, 3316–3324.
- 303 X. Wang, C. Wang, L. Cheng, S. T. Lee and Z. Liu, *J. Am. Chem. Soc.*, 2012, **134**, 7414–7422.
- 304 S. A. Khan, R. Kanchanapally, Z. Fan, L. Beqa, A. K. Singh, D. Senapati and P. C. Ray, *Chem. Commun.*, 2012, **48**, 6711–6713.
- 305 M. Y. Sha, H. Xu, M. J. Natan and R. Cromer, *J. Am. Chem. Soc.*, 2008, **130**, 17214–17215.
- 306 P. Zhang, R. Zhang, M. Gao and X. Zhang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 370–376.
- 307 X. Wang, X. Qian, J. J. Beitler, Z. G. Chen, F. R. Khuri, M. M. Lewis, H. J. C. Shin, S. Nie and D. M. Shin, *Cancer Res.*, 2011, **71**, 1526–1532.
- 308 X. Wu, L. Luo, S. Yang, X. Ma, Y. Li, C. Dong, Y. Tian, L. Zhang, Z. Shen and A. Wu, *ACS Appl. Mater. Interfaces*, 2015, **7**, 9965–9971.
- 309 W. Shi, R. J. Paproski, R. Moore and R. Zemp, *J. Biomed. Opt.*, 2014, **19**, 56014.
- 310 T. Xue, S. Wang, G. Ou, Y. Li, H. Ruan, Z. Li, Y. Ma, R. Zou, J. Qiu, Z. Shen and A. Wu, *Anal. Methods*, 2019, **11**, 2918–2928.
- 311 Y. S. Borghei, M. Hosseini, M. Dadmehr, S. Hosseinkhani, M. R. Ganjali and R. Sheikhejad, *Anal. Chim. Acta*, 2016, **904**, 92–97.
- 312 C. D. Medley, J. E. Smith, Z. Tang, Y. Wu, S. Bamrungsap and W. Tan, *Anal. Chem.*, 2008, **80**, 1067–1072.
- 313 W. Lu, S. R. Arumugam, D. Senapati, A. K. Singh, T. Arbnesi, S. A. Khan, H. Yu and P. C. Ray, *ACS Nano*, 2010, **4**, 1739–1749.
- 314 W. Liu, H. Wei, Z. Lin, S. Mao and J. M. Lin, *Biosens. Bioelectron.*, 2011, **28**, 438–442.
- 315 E. I. Galanzha, E. V. Shashkov, T. Kelly, J. W. Kim, L. Yang and V. P. Zharov, *Nat. Nanotechnol.*, 2009, **4**, 855.
- 316 D. S. Seferos, D. A. Giljohann, H. D. Hill, A. E. Prigodich and C. A. Mirkin, *J. Am. Chem. Soc.*, 2007, **129**, 15477–15479.
- 317 C. H. J. Choi, L. Hao, S. P. Narayan, E. Auyeung and C. A. Mirkin, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 7625–7630.
- 318 T. L. Halo, K. M. McMahon, N. L. Angeloni, Y. Xu, W. Wang, A. B. Chinen, D. Malin, E. Strekalova, V. L. Cryns, C. Cheng, C. A. Mirkin and C. S. Thaxton, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 17104–17109.
- 319 W. E. Briley, M. H. Bondy, P. S. Randeria, T. J. Dupper and C. A. Mirkin, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 9591–9595.
- 320 M. Toloudi, P. Apostolou, M. Chatziioannou and I. Papsotiriou, *Case Rep. Oncol.*, 2011, **4**, 44–54.
- 321 K. Lee, V. P. Drachev and J. Irudayaraj, *ACS Nano*, 2011, **5**, 2109–2117.
- 322 M. A. Woo, S. M. Lee, G. Kim, J. H. Baek, M. S. Noh, J. E. Kim, S. J. Park, A. Minai-Tehrani, S. C. Park, Y. T. Seo,



- Y. K. Kim, Y. S. Lee, D. H. Jeong and M. H. Cho, *Anal. Chem.*, 2009, **81**, 1008–1015.
- 323 G. Van Niel, I. Porto-Carreiro, S. Simoes and G. Raposo, *J. Biochem.*, 2006, **140**, 13–21.
- 324 E. van der Pol, A. N. Boing, P. Harrison, A. Sturk and R. Nieuwland, *Pharmacol. Rev.*, 2012, **64**, 676–705.
- 325 J. M. Street, P. E. Barran, C. L. Mackay, S. Weidt, C. Balmforth, T. S. Walsh, R. T. A. Chalmers, D. J. Webb and J. W. Dear, *J. Transl. Med.*, 2012, **10**, 5.
- 326 C. Lässer, V. Seyed Alikhani, K. Ekström, M. Eldh, P. Torregrosa Paredes, A. Bossios, M. Sjöstrand, S. Gabrielsson, J. Lötvall and H. Valadi, *J. Transl. Med.*, 2011, **9**, 9.
- 327 P. A. Gonzales, T. Pisitkun, J. D. Hoffert, D. Tchapyjnikov, R. A. Star, R. Kleta, N. S. Wang and M. A. Knepper, *J. Am. Soc. Nephrol.*, 2009, **20**, 363–379.
- 328 M. P. Caby, D. Lankar, C. Vincendeau-Scherrer, G. Raposo and C. Bonnerot, *Int. Immunol.*, 2005, **17**, 879–887.
- 329 Z. Wang, H. J. Wu, D. Fine, J. Schmulen, Y. Hu, B. Godin, J. X. J. Zhang and X. Liu, *Lab Chip*, 2013, **13**, 2879–2882.
- 330 B. Gyorgy, K. Modos, E. Pallinger, K. Paloczi, M. Pasztoi, P. Misjak, M. A. Deli, A. Sipos, A. Szalai, I. Voszka, A. Polgar, K. Toth, M. Csete, G. Nagy, S. Gay, A. Falus, A. Kittle and E. I. Buzas, *Blood*, 2011, **117**, e39–e48.
- 331 M. C. Larson, M. R. Luthi, N. Hogg and C. A. Hillery, *Cytometry, Part A*, 2013, **83**, 242–250.
- 332 R. A. Dragovic, C. Gardiner, A. S. Brooks, D. S. Tannetta, D. J. P. Ferguson, P. Hole, B. Carr, C. W. G. Redman, A. L. Harris, P. J. Dobson, P. Harrison and I. L. Sargent, *Nanomedicine*, 2011, **7**, 780–788.
- 333 F. Momen-Heravi, L. Balaj, S. Alian, P. Y. Mantel, A. E. Halleck, A. J. Trachtenberg, C. E. Soria, S. Oquin, C. M. Bonebreak, E. Saracoglu, J. Skog and W. P. Kuo, *Biol. Chem.*, 2013, **394**, 1253–1262.
- 334 H. G. Lamparski, A. Metha-Damani, J. Y. Yao, S. Patel, D. H. Hsu, C. Ruegg and J. B. Le Pecq, *J. Immunol. Methods*, 2002, **270**, 211–226.
- 335 A. Liga, A. D. B. Vliegthart, W. Oosthuyzen, J. W. Dear and M. Kersaudy-Kerhoas, *Lab Chip*, 2015, **15**, 2388–2394.
- 336 C. Chen, J. Skog, C. H. Hsu, R. T. Lessard, L. Balaj, T. Wurdinger, B. S. Carter, X. O. Breakefield, M. Toner and D. Irimia, *Lab Chip*, 2010, **10**, 505–511.
- 337 A. Clayton, J. Court, H. Navabi, M. Adams, M. D. Mason, J. A. Hobot, G. R. Newman and B. Jasani, *J. Immunol. Methods*, 2001, **247**, 163–174.
- 338 B. J. Tauro, D. W. Greening, R. A. Mathias, H. Ji, S. Mathivanan, A. M. Scott and R. J. Simpson, *Methods*, 2012, **56**, 293–304.
- 339 E. Van Der Pol, A. G. Hoekstra, A. Sturk, C. Otto, T. G. Van Leeuwen and R. Nieuwland, *J. Thromb. Haemost.*, 2010, **8**, 2596–2607.
- 340 R. Lacroix, S. Robert, P. Poncelet, R. S. Kasthuri, N. S. Key and F. Dignat-George, *J. Thromb. Haemostasis*, 2010, **8**, 2571–2574.
- 341 E. N. M. Nolte-t Hoen, E. J. van der Vlist, M. Aalberts, H. C. H. Mertens, B. J. Bosch, W. Bartelink, E. Mastrobattista, E. V. B. van Gaal, W. Stoorvogel, G. J. A. Arkesteijn and M. H. M. Wauben, *Nanomedicine*, 2012, **8**, 712–720.
- 342 L. Tirinato, F. Gentile, D. Di Mascolo, M. L. Coluccio, G. Das, C. Liberale, S. A. Pullano, G. Perozziello, M. Francardi, A. Accardo, F. De Angelis, P. Candeloro and E. Di Fabrizio, *Microelectron. Eng.*, 2012, **97**, 337–340.
- 343 L. T. Kerr, L. Gubbins, K. W. Gorzel, S. Sharma, M. Kell, A. McCann and B. M. Hennelly, *Biophotonics: Photonic Solutions for Better Health Care IV*, International Society for Optics and Photonics, 2014, vol. 9129, pp. 91292Q–1–91292Q–9.
- 344 Z. Wang, S. Zong, Y. Wang, N. Li, L. Li, J. Lu, Z. Wang, B. Chen and Y. Cui, *Nanoscale*, 2018, **10**, 9053–9062.
- 345 C. Lee, R. P. Carney, S. Hazari, Z. J. Smith, A. Knudson, C. S. Robertson, K. S. Lam and S. Wachsmann-Hogiu, *Nanoscale*, 2015, **7**, 9290–9297.
- 346 Y. G. Zhou, R. M. Mohamadi, M. Poudineh, L. Kermanshah, S. Ahmed, T. S. Safaei, J. Stojcic, R. K. Nam, E. H. Sargent and S. O. Kelley, *Small*, 2016, **12**, 727–732.
- 347 H. Im, H. Shao, Y. I. Park, V. M. Peterson, C. M. Castro, R. Weissleder and H. Lee, *Nat. Biotechnol.*, 2014, **32**, 490.
- 348 G. Di Noto, A. Bugatti, A. Zandrini, E. L. Mazzoldi, A. Montanelli, L. Caimi, M. Rusnati, D. Ricotta and P. Bergese, *Biosens. Bioelectron.*, 2016, **77**, 518–524.
- 349 S. A. Melo, H. Sugimoto, J. T. O'Connell, N. Kato, A. Villanueva, A. Vidal, L. Qiu, E. Vitkin, L. T. Perelman, C. A. Melo, A. Lucci, C. Ivan, G. A. Calin and R. Kalluri, *Cancer Cell*, 2014, **26**, 707–721.
- 350 S. S. Kanwar, C. J. Dunlay, D. M. Simeone and S. Nagrath, *Lab Chip*, 2014, **14**, 1891–1900.
- 351 C. Théry, S. Amigorena, G. Raposo and A. Clayton, *Curr. Protoc. Cell Biol.*, 2006, **30**, 3–22.
- 352 M. Czarnek and J. Bereta, *Sci. Rep.*, 2017, **7**, 11682.

