A new biomolecular quantitation method, nanoparticle-mediated photothermal bioassay, using a common thermometer as the signal reader was developed. Using an immunoassay as a proof of concept, iron oxide nanoparticles (NPs) captured in the sandwich-type assay system were transformed into a near-infrared (NIR) laser-driven photothermal agent, Prussian blue (PB) NPs, which acted as a photothermal probe to convert the assay signal into heat through the photothermal effect, thus allowing sensitive biomolecular quantitation using a thermometer. This is the first report of biomolecular quantitation using a thermometer and also serves as the first attempt to introduce the nanoparticle-mediated photothermal effect for bioassays.

The development of new methods for quantitative detection of biomolecules has been the subject of great research interest especially for disease biomarker detection in clinical diagnosis. A number of immunoassays based on different detection principles, such as the traditional enzyme linked immunosorbent assay (ELISA), surface plasmon resonance, surface enhanced Raman scattering, chemiluminescence, and electrochemistry and fluorescence methodologies, have been widely used in biomolecular detection. Despite great research progress, these traditional bioassays usually have several limitations, especially in resource-limited settings. Typically, one of the most critical bottlenecks is the assay readout method, because most traditional readout methods rely on bulky and expensive analytical equipment. Colorimetric results can be observed by the naked eye for qualitative analysis or semi-quantitative analysis, but the sensitivity of this detection is low. Further aid of other analytical, imaging and computation equipment is required to achieve quantitative detection. Furthermore, professionally trained personnel to use the equipment, software and assay protocols are generally indispensable during these conventional bioassays, further limiting their potential for wide application. Therefore, the development of new cost-effective readout methods for quantitative detection of various biomolecules is in great demand to advance affordable biomolecular quantitation and to address limitations of current methods to improve global health.

The study of nanoparticle-mediated photothermal effects has currently emerged as a particularly attractive research topic in various fields because of the unique light-to-heat photo-physical conversion property. In particular, the near-infrared (NIR) light-driven photothermal effect has shown great promise in the biomedical field for non-invasive photothermal therapy of cancers employing heat converted by photothermal agents from NIR light absorption. A variety of nanomaterials, such as Prussian blue (PB)-, carbon- and gold-based nanomaterials that can convert the NIR light into heat, have been developed as photothermal therapeutic agents. It is worth noting that heat generated from the photothermal therapeutic process can be accurately monitored using a thermometer, one of the most widely-used, portable and inexpensive analytical tools. Therefore, introduction of the nanoparticle-mediated photothermal effect in bioassays makes it feasible to develop a novel low-cost approach for biomolecular quantitation using a common thermometer. Although the photothermal effect was extensively studied for photothermal therapy, to the best of our knowledge, the nanoparticle-mediated photothermal effect has never been utilized for quantitative biomolecular detection.

Herein, we have introduced the nanoparticle-mediated photothermal effect to develop a new biomolecular quantitation method, nanoparticle-mediated photothermal bioassay, using a common thermometer as the signal reader for quantitative biomolecular detection. As shown in Fig. 1, using a typical sandwich-type immunoassay as the proof of concept,
monoclonal antibody was used as the capture antibody pre-immobilized on microcentrifuge tube surface, whereas the iron oxide nanoparticles (Fe₃O₄ NPs)-labelled polyclonal antibody was used as the detection antibody. Different concentrations of cancer biomarkers (antigens) were used as the target analytes. After the incubation with antigen and thorough washing, Fe₃O₄ NPs were introduced to form a typical sandwich structure. Because the photothermal effect of Fe₃O₄ NPs was weak, we converted Fe₃O₄ NPs into Prussian blue (PB) NPs for a stronger photothermal effect. Fe₃O₄ NPs captured in the sandwich-type immunoassay system were then dissolved in acidic conditions to release ferric ions, followed by reactions with potassium ferrocyanide to produce PB NPs, a NIR laser-driven photothermal agent. The as-obtained PB NPs acted as a highly sensitive photothermal probe to convert the immunoassay signal into heat through the NIR laser-driven photothermal effect, thus allowing sensitive and quantitative readout of the immunoassay using a common thermometer. Using prostate-specific antigen (PSA) as the analyte, the photothermal effect, specificity and reliability of the developed photothermal immunoassay were studied systematically. To the best of our knowledge, this is the first attempt to introduce the nanoparticle-mediated photothermal effect for biomolecule quantitation. Most importantly, the innovative application of the nanoparticle-mediated photothermal effect has enabled a new biomolecular quantitation strategy using a common thermometer, thus providing new opportunities toward advances in the development of affordable bioassays, particularly in low-resource settings.

PB as an ancient dye is a prototype of mixed-valence transition metal hexacyanoferrates.²⁷ Due to their strong optical absorption in the NIR region and high photothermal efficiency, recently, PB NPs have been explored as a new generation of NIR laser-driven photothermal agent.¹⁵,²⁸

To confirm the production of PB NPs in the immunoassay solution after the nanoparticle transformation process, UV-Vis spectroscopic characterization was carried out before and after the reaction with potassium ferrocyanide. As expected, a clear color change of the immunoassay solution to blue was observed at a 32.0 ng mL⁻¹ PSA concentration after the nanoparticle transformation process (Fig. 2A), corresponding to the typical blue color of PB. Furthermore, a broad absorption peak at 748 nm in the UV-Vis spectra of the immunoassay solution (32.0 ng mL⁻¹ PSA) was observed after the transformation process (Fig. 2B), whereas no absorption peak was exhibited before the process. The absorption peak corresponded well with that of PB NPs due to the charge transfer transition between Fe(II) and Fe(III) in PB,¹⁵,¹⁶ indicating the generation of PB in the immunoassay solution after the nanoparticle transformation process. The slight red shift of the absorption peak of PB in the immunoassay solution might be attributed to the different matrix effect from the immunoassay solution. In addition, no apparent absorption peak (Fig. 2B) and color change (Fig. 2A) were observed even after the nanoparticle transformation process in the absence of target PSA because no Fe₃O₄ NPs were captured in the sandwich-type immunoassay system in the absence of target PSA, and thus no PB
would be generated in the control solutions. These results confirmed the production of PB in the immunoassay solution.

To further confirm the production of PB NPs in the immunoassay solution, transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR, see Fig. S1 in the ESI†) were performed to characterize the changes in the nanoparticles in the immunoassay solution at a 32.0 ng mL\(^{-1}\) PSA concentration before and after the nanoparticle transformation process. Fe\(_3\)O\(_4\) NPs captured in the sandwich-type immunoassay system were collected for TEM observation prior to the dissolution procedure in the acidic condition. Nanoparticles with uniformly spherical morphology and an average diameter of 40 nm were observed in the TEM image (Fig. 2C), which corresponded well with the product information from the manufacturer (Ocean NanoTech LLC, USA). However, an obvious change in morphology was observed after the nanoparticle transformation process (Fig. 2C). With the disappearance of the spherical iron oxide NPs, nanoparticles with clear cubic morphology in the size range from 20 to 100 nm were observed in the TEM image, which was in good agreement with the well-known cubic morphology of PB NPs\(^{15,29,30}\). The result further confirmed the successful Fe\(_3\)O\(_4\)-to-PB NPs transformation in the immunoassay solution.

To investigate the feasibility of PB NPs for photothermal immunoassay, NIR laser-driven photothermal effect of PB NPs was first studied. Using water as the control, aqueous dispersions (1.0 mL) of different concentrations of PB NPs were irradiated by an 808 nm laser for 10 min at a power density of 3.12 W cm\(^{-2}\). A pen-style digital thermometer was used to monitor the temperature of the dispersions during the irradiation process. Fig. 3A shows the temperature change of the dispersions during the process. The PB NPs dispersions showed a dramatic temperature increase during the irradiation process, whereas no significant temperature change was observed for water. It could be found that the higher concentrations of PB NPs ranging from 0.0125 to 0.0375 mg mL\(^{-1}\), recorded higher temperatures. PB NPs at a low concentration of 0.0125 mg mL\(^{-1}\) could lead to a temperature increase of 11.3 °C. Surprisingly, the 0.0375 mg mL\(^{-1}\) PB NPs dispersion reached a high temperature increase up to 36.3 °C after the irradiation process. Moreover, irradiation for only 1.0 min can result in a rapid temperature increase of 10.0 °C at 0.0375 mg mL\(^{-1}\). The result demonstrated a considerable photothermal effect of PB NPs upon the irradiation with a NIR laser, which is attributed to their strong optical absorption in the NIR region due to the charge transfer transition between Fe(II) and Fe(III) in PB NPs.\(^{31}\)

To evaluate the photothermal effect before and after the Fe\(_3\)O\(_4\)-to-PB NPs transformation process, the photothermal effect of Fe\(_3\)O\(_4\) NPs was studied in comparison with that of PB NPs. Fig. 3B shows the temperature change of the same concentration (0.025 mg mL\(^{-1}\)) of Fe\(_3\)O\(_4\) NPs and PB NPs during the irradiation process (808 nm, 3.12 W cm\(^{-2}\)) for 10 min. As can be observed, Fe\(_3\)O\(_4\) NPs showed a minor temperature increase of 4.3 °C after the irradiation, indicating a weak photothermal effect of Fe\(_3\)O\(_4\) NPs upon NIR laser irradiation. However, PB NPs showed a 5.2-fold higher temperature increase (22.5 °C) than the Fe\(_3\)O\(_4\) NPs, which indicated a much stronger photothermal effect of PB NPs compared to the Fe\(_3\)O\(_4\) NPs. Therefore, the stronger photothermal effect obtained from the Fe\(_3\)O\(_4\)-to-PB NPs transformation process makes it feasible to use a common thermometer for high-sensitivity photothermal detection. This is the main reason that we transformed Fe\(_3\)O\(_4\) NPs to PB NPs in the immunoassay.

To demonstrate the feasibility of the photothermal strategy for quantitative analysis, the relationship between the photothermal effect-induced temperature increase and the concentration of PB NPs was studied. Aqueous dispersions (0.15 mL) of different concentrations of PB NPs were exposed to the laser
(5.26 W cm\(^{-2}\)) for 1.5 min. Fig. 3C shows different temperature increases (\(\Delta T\)) \textit{versus} different concentrations of PB NPs and Fe\(_3\)O\(_4\) NPs. As the PB NPs concentration increased, the temperature increased dramatically after the irradiation. Significantly, the temperature increase exhibited a linear relationship with concentrations ranging from 0.00156 to 0.0250 ng mL\(^{-1}\) with the square of correlation coefficient of 0.99. Furthermore, temperature measurement of eight 0.0125 ng mL\(^{-1}\) PB NPs dispersions showed a low relative standard deviation (RSD) of 2.16%, implying good reproducibility of the thermometer for readout of the photothermal effect. For comparison, the same concentrations of Fe\(_3\)O\(_4\) NPs were also irradiated to study the temperature elevation. Although Fe\(_3\)O\(_4\) NPs showed a linear relationship between the temperature increase and its concentration, only a 4.0-fold lower sensitivity than that of PB NPs was achieved due to the weak photothermal effect of Fe\(_3\)O\(_4\) NPs. Overall, this systematically studied series indicated the great potential of the photothermal strategy for quantitative detection of PSA and other bioassays using a common thermometer as the assay signal reader.

On such a basis, a complete photothermal immunoassay was performed with a common thermometer as the signal reader as illustrated in Fig. 1. After the Fe\(_3\)O\(_4\)-to-PB NPs transformation procedure, different immunoassay solutions obtained from different concentrations of PSA in the range from 1.0 to 64.0 ng mL\(^{-1}\) were irradiated (5.26 W cm\(^{-2}\)) for 1.5 min, and the photothermal effect-induced temperature increase was measured using the pen-style digital thermometer. Fig. 4A shows the temperature increase of the immunoassay solutions before and after the Fe\(_3\)O\(_4\)-to-PB NPs transformation as a function of PSA concentration. As the PSA concentration increased, a dramatic increase in temperature was observed after irradiation. An extraordinarily high temperature increase of 38.3 °C from 64.0 ng mL\(^{-1}\) PSA was measured, whereas an obvious temperature increase of 1.8 °C was observed even at 1.0 ng mL\(^{-1}\) PSA, revealing high sensitivity of the PB NPs-based photothermal immunoassay (after nanoparticle transformation). However, similar to Fig. 3C, only minor temperature increases were recorded from the Fe\(_3\)O\(_4\) NPs-based photothermal immunoassay (before nanoparticle transformation) even at the high concentration of 64.0 ng mL\(^{-1}\) PSA (6.0 °C).

It was also found from Fig. 4A that the temperature increases from both Fe\(_3\)O\(_4\) and PB NPs-based photothermal immunoassays were proportional to the logarithm of PSA concentrations in the range from 2.0 to 64.0 ng mL\(^{-1}\) with the squares of correlation coefficients of 0.99 \((Y \sim \log C_{PSA} (\text{ng mL}^{-1}) + 0.245)\) and 0.98 \((Y \sim 23.3 \log C_{PSA} (\text{ng mL}^{-1}) - 5.08)\), respectively. Therefore, a 7.4-fold higher slope was obtained as a result of the Fe\(_3\)O\(_4\)-to-PB NPs transformation. With only a common thermometer for quantitative readout in the photothermal immunoassay, PSA can be determined at the limit of detection (LOD) of 1.0 ng mL\(^{-1}\) without the aid of any advanced analytical equipment. Although this concentration is relatively higher than that of some traditional methods, such as electrochemical and fluorescent methods,\(^{32-34}\) it is comparable to the conventional ELISA method (LOD: 1.0 ng mL\(^{-1}\)) and commercial PSA ELISA kits (LOD: 1.0 ng mL\(^{-1}\), Biocell Bio-technol. Co., Ltd, Zhengzhou, China) using UV-Vis spectrometers, as reported previously in the literature.\(^{35}\) In addition, it is worth noting that the developed photothermal immunoassay can completely meet the requirements for clinical prostate cancer diagnostics, because the threshold concentration of the total PSA in human serum in prostate cancer diagnostics is 4.0 ng mL\(^{-1}\).\(^{35,36}\)

To evaluate the specificity of the developed photothermal immunoassay, some common interfering substances, including carcinoembryonic antigen (CEA), immunoglobulin G (IgG), hepatitis B surface antigen (HBsAg) and bovine serum albumin (BSA), were tested. As shown in Fig. 4B, a high temperature increase of 23.9 °C was observed from target PSA (16.0 ng mL\(^{-1}\)), whereas no significant temperature increase was observed from other interfering substances even with 10-fold higher concentrations, indicating high specificity of our photothermal immunoassay. Along with the specificity, the reproducibility of the photothermal immunoassay was studied by measuring the temperature elevation of six immunoassay solutions obtained from the same PSA concentration (64.0 ng mL\(^{-1}\)). The RSD was 5.19%, indicating acceptable reproducibility of the developed photothermal immunoassay.

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*Fig. 4* (A) Calibration plot of temperature increase (\(\Delta T\)) vs. logarithm of PSA concentration from immunoassay solutions before and after the Fe\(_3\)O\(_4\)-to-PB NPs transformation process. (B) Specificity test of the photothermal immunoassay method with a thermometer as the signal reader. The concentrations of BSA, CEA, IgG, HBsAg and PSA are 16 \(\mu\)g mL\(^{-1}\), 160 ng mL\(^{-1}\), 160 ng mL\(^{-1}\), 160 ng mL\(^{-1}\) and 16.0 ng mL\(^{-1}\), respectively.
To validate the analytical reliability of the developed photothermal immunoassay in the detection of real human samples, normal human serum samples were spiked with different concentrations of PSA for photothermal determinations. The recoveries of target PSA spiked in three serum samples were estimated using the thermometer-based readout method. Table S1† shows that all the percent recoveries fell in the range of 91.7%–95.8%. These recoveries are comparable to those of some standard commercial PSA ELISA kits (e.g. 94–112% from Abcam, USA and 95–100% from USBio, USA) according to their product information. These results demonstrated an acceptable analytical reliability of the developed photothermal immunoassay for detection of real human serum samples.37

Conclusions

In summary, based on the nanoparticle-mediated photothermal effect, we have developed a new photothermal biomolecular quantitation method using a common thermometer as the quantitative signal reader. Although the nanoparticle-mediated photothermal effect has been extensively studied in disease therapy, this study serves as the first attempt to introduce it in bioassays for quantitation of various disease biomarkers and proteins. The thermometer-based readout method is not only low-cost, portable and widely-available, but also requires minimal professional training in the use of a thermometer and in data readout, without the need of any specialized software for equipment control and data processing. Furthermore, given that small, lightweight, powerful and portable NIR laser systems are becoming commercially available, some handheld laser pointers could be used as the light source in our photothermal detection method for a point of care application. Most importantly, the innovative application of the nanoparticle-mediated photothermal effect will provide new opportunities toward advances in affordable biomolecular detection possibly even by non-professional people in various public venues, especially in low-resource settings such as developing nations. We envision that the photothermal biomolecular detection strategy will have broad applications ranging from clinical disease diagnosis to various biochemical analyses.

Acknowledgements

We would like to acknowledge the financial support from the National Institute of General Medical Sciences of the NIH (SC2GM105584) and the National Institute of Allergy and Infectious Disease of the NIH (R21AI107415). Financial support from NIH RCMI for the BBRC Pilot grant, the University of Texas at El Paso (UTEP) for the IDR Pilot grant, Multidisciplinary Research Award Program (MRAP) and URI Program, University of Texas (UT) System for the STARS award, is also greatly acknowledged. We are also grateful to the support from Dr Chuan Xiao’s Group at UTEP for the UV-Vis spectroscopic measurement and Dr Bernal’s group at UTEP for TEM images.

Notes and references