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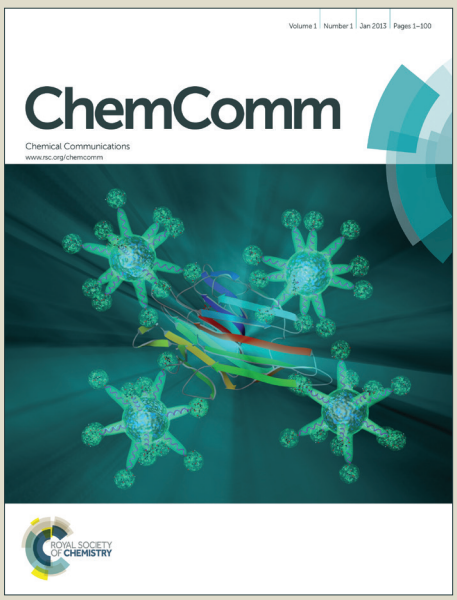

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ARTICLE TYPE

Polypyrrole Nanoprobes with Low Non-specific Protein Adsorption for Intracellular mRNA Detection and Photothermal Therapy

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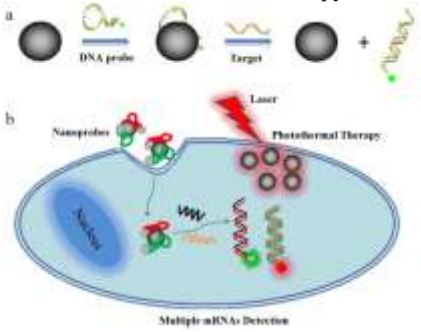
In this work, we discovered that polypyrrole nanoparticles (PPy NPs) displayed a low non-specific protein adsorption. We herein present the first PPy NPs-based biosensing platform for intracellular mRNAs detection in living cells. We also demonstrate that PPy NPs exhibit high NIR absorbance and can be utilized for cancer photothermal therapy.

Messenger RNA (mRNA), a single-stranded ribonucleic acid with genetic information, is the blueprint for the cellular production of proteins. Some mRNAs are disease-relevant and can be utilized as markers to determine the stage of the disease, such as cancer.[1] Thus, the study of mRNA is critical for understanding basic biology and identifying therapeutic and diagnostic targets. Over the past few years, several techniques have been developed for mRNA detection, including microarray analysis,[2] real-time polymerase chain reaction (RT-PCR)[3] and northern blot.[4] However, these methods are available for detecting relative mRNA expression in bulk samples, but they are incapable of detecting cell-to-cell variations. More importantly, many physical and biological processes dependent not just on bulk mRNA expression, but are also highly related with cell-to-cell variations in mRNA.[5, 6] Therefore, it is highly necessary to develop useful approaches for mRNA detection in living cells. Up to now, alternative methods have been established to analysis endogenous mRNAs.[7,8] Among these, nanoparticle (NP)-based methods have attracted great attention due to their many attractive characteristics, such as high sensitivity, quick response, high efficiency and cost effectiveness. Several NPs have been used for intracellular mRNA detection, such as gold nanoparticles (AuNPs),[9,10] SnO_2 nanoparticles (SnO_2 NPs)[11] and cobalt ferrite magnetic nanoparticles (Co ferrite MNPs).[12] However, it is complex and time-consuming for the covalent attachment of oligonucleotides probes to NPs. On the other hand, many NPs can directly adsorb oligonucleotides to form stable nanoprobes, such as grapheme oxide (GO),[13,14] single-walled carbon nanotubes (SWCNTs),[15] WS_2 nanosheet,[16] carbon nitride nanosheet,[17] MoS_2 nanosheet[18] and MnO_2 nanosheet.[19] Albeit substantial progress was accomplished *in vitro*, the non-specific protein adsorption on NPs limits their applications in living cells.

Protein adsorption is the first response from human body to foreign materials exposed to physical environment. The non-specific adsorption of biomolecules such as proteins and peptides from biological media on the surface of NPs is a big challenge to achieve ultrasensitive nanosensing in living cells and *in vivo*.[20] The system suffers from high background and low sensitivity. Moreover, most of non-specific protein adsorption in living body is harmful, which may induce adverse bioresponses, including complement activation, coagulation and thrombosis, or other undesirable biophysical and biochemical processes.[21,22] Therefore, the development of NPs with low non-specific protein adsorption is desirable for bioassays in living cells and *in vivo*.

Polypyrrole (PPy), an organic conductive polymer, has received great attention in bioelectronics and biomedical application due to its inherent features, including high conductivity, outstanding stability and good biocompatibility.[23,24] Photothermal therapy (PTT) is able to burn cancer through converting near-infrared (NIR) light energy into heat by using NIR-absorbing agents. Compared with traditional cancer therapies such as radiotherapy and chemotherapy, PTT cancer treatment is a minimally invasive or noninvasive technique.[25] With strong NIR absorbance, PPy NPs could serve as strong photothermal agents, showing excellent cancer ablation effect both *in vitro* and *in vivo*.[26-28] In addition, PPy NPs have been reported as strong quenchers of various fluorescent dyes over a wide wavelength range.[29] The properties mentioned above make this polymer extremely useful for the designing of various biosensors. Recently, we discovered that PPy NPs displayed a low non-specific protein adsorption compared to other nanomaterials, even in high concentrations of protein. Inspired by this observation, we herein present the first PPy NPs-based biosensing platform for intracellular multiple mRNAs detection in living cells. The PPy NPs can directly adsorb dye-labeled hairpin DNA to form PPy-DNA nanoprobes and effectively quench the fluorescence of the dyes. In the presence of the target, the specific binding between the DNA probe and its target induces the formation of a duplex structure, resulting in the release of the DNA probes from PPy NPs and subsequent recovery of the fluorescence (Scheme 1a). Additionally, PPy NPs can act as a common fluorescence quencher toward different kinds of fluorescent dyes. Thus, the proposed method could be expanded easily to detect multiple mRNAs simultaneously. Since many diseases and biological processes involve changes in the expression of multiple genes, such a multiplexed assay can provide important information on genetic interactions in these complex systems. Meanwhile, due to the NIR absorption and good photostability, PPy NPs can be employed for cancer photothermal therapy (PPT) (Scheme 1b). Our results revealed that PPy NPs hold great promise as candidates for intracellular

multiple mRNAs detection and cancer therapy.



Scheme 1 (a) PPy-DNA nanoprobes for mRNA detection. (b) Illustration of the PPy-DNA nanoprobes for intracellular mRNA detection and photothermal therapy.

PPy NPs were synthesized according to a previous report with minor modifications.[30] The TEM images indicated that the as-obtained PPy NPs are spherical-like, and the average diameter of these nanoparticles was approximately 30 nm (Fig. 1a). The average hydrodynamic diameter of PPy NPs was about 34 nm obtained by dynamic light scattering (DLS) (Fig. S1). It's also worth pointing out that the as-obtained PPy NPs still dispersed well in water and physiological solution over three months (Fig. S2). Furthermore, as the UV-vis-NIR absorption spectra shown in Fig. 1b, the as-obtained PPy NPs displayed a broad absorption band in the range of 400-1000 nm, which made it a good quencher for varieties of fluorescence dyes and a good agent for cancer photothermal therapy.

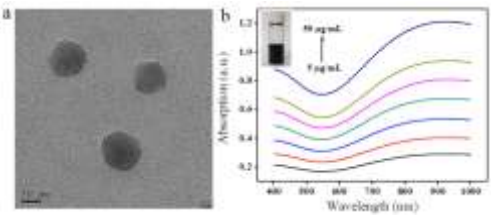


Fig. 1 (a) TEM image of PPy NPs. (b) UV-vis-NIR absorption spectra of PPy NPs with different concentrations (form bottom to top: 5, 10, 15, 20, 30, 40 and 50 μg/mL).

To test the feasibility of PPy-DNA nanoprobes for mRNA detection, an RNA sequence associated with cellular myelocytomatosis oncogene (c-myc) is utilized as a model target mRNA. All the sequences used in this work are shown in Table S1. The interaction between PPy NPs and hairpin DNA was possibly led by non-covalent binding such as hydrogen bonding, π-π stacking, and charge-transfer complexes between the units of PPy NPs and nucleobases.[31-33] As expected, the fluorescence of nanoprobes was strongly quenched (Fig. S3a). With the increasing concentration of PPy NPs, the fluorescence intensity of nanoprobes decreased and trended to a minimum value at 50 μg/mL PPy NPs (Fig. S3a). Thus, 50 μg/mL PPy NPs was used for analytical purposes. The quenching efficiency (Q_E, [%]) of PPy NPs was up to 98%, which is comparable to that of AuNPs,[34] GO[35] and SWCNTs.[36] The higher quenching efficiency would lead to a higher signal-to-background ratio and thus better sensitivity and a greater dynamic range for target detection.

Next, we investigated the properties of the nanoprobes for the detection of mRNA target. As expected, the nanoprobes were specifically bound to their mRNA targets and generated a significant fluorescence recovery (Fig. 2a). The fluorescence intensity of the nanoprobes increased with increasing concentration of the mRNA targets from 0 to 200 nM (Fig. S3b). By comparison, the signals had a negligible change in the presence of non-complementary targets and were comparable to background fluorescence (Fig. 2a).

Non-specific adsorption of biomolecules such as proteins and peptides from biological media on the surface of NPs is a serious problem in many bioapplications, which leads to high background and false signals. Therefore, nanoprobes with low non-specific protein adsorption are desirable for nanosensing in complex media, such as whole blood and high concentration serum or plasma.[37] Herein, we investigated the non-specific protein adsorption on the PPy-DNA nanoprobes. Carbon-based nanomaterials were chosen as comparison. Carbon-based nanomaterials, such as GO and SWCNTs, have attracted considerable interest in nanosensing owing to their unique properties.[38,39] As shown in Fig. 2b, the fluorescence showed a negligible enhancement after the PPy-DNA nanoprobes were treated with DMEM culture medium. In contrast, when GO-DNA or SWCNTs-DNA nanoprobes were under the same condition, the fluorescence displayed an obvious recovery (Fig. 2c, 2d).

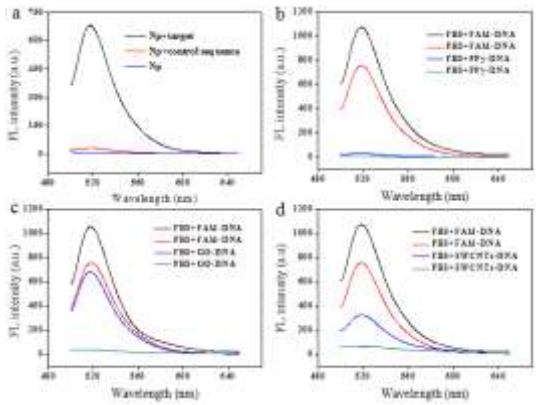


Fig. 2 (a) Fluorescence emission spectra of PPy-DNA nanoprobes (containing 50 μg/mL PPy NPs and 50 nM FAM-DNA) incubated with 200 nM complementary target and control sequence for 30 min. (b) Fluorescence emission spectra of FAM-DNA (50 nM) or PPy-DNA nanoprobes dispersed in DMEM culture medium (containing 10% FBS) or PBS. (c) Fluorescence emission spectra of FAM-DNA or GO-DNA nanoprobes dispersed in DMEM culture medium or PBS. (d) Fluorescence emission spectra of FAM-DNA or SWCNTs-DNA nanoprobes dispersed in DMEM culture medium or PBS.

To further estimate the non-specific adsorption on PPy NPs, bovine serum albumin (BSA) was employed as model protein. As shown in Fig. S4a, when PPy-DNA nanoprobes were incubated with BSA, the fluorescence signal showed a negligible change even at the concentration of 10 mg/mL. In contrast, both the fluorescence signal of GO-DNA and SWCNTs-DNA nanoprobes displayed significantly enhanced after treated with 2 mg/mL of BSA (Fig. S4b, S4c). This result was consistent with previous studies, where carbon-based nanoprobes showed high non-specific desorption in the complex medium.[40,41] Compared with carbon-based nanoprobes, PPy-DNA nanoprobes showed much lower non-specific protein adsorption. We presume lower non-specific protein adsorption that may be due to the strong hydrophilicity of PPy NPs functionalized with PVP, which could suppress non-specific protein adsorption.[42] However, the detailed

interaction mechanism is still not fully understood.

The PPy-DNA nanoprobes were then applied to detect mRNA in living cells. C-myc is a potent activator of tumorigenesis, and it is deregulated in many cancers. MCF-7 cells, which have a high expression of c-myc, were chosen as target cancer cells, whereas MCF-10A cells are deficient in c-myc expression were used as control.[43] When MCF-7 cells was incubated with the nanoprobes, a strong green fluorescence signal for c-myc mRNA was observed under confocal laser scanning microscopy (CLSM) (Fig. 3a). In comparison, the fluorescence signal was unobservable after MCF-10A cells were incubated with nanoprobes. Moreover, overlay of fluorescence images and bright-field images showed that fluorescence signal were localized in the cytoplasm of cells, suggesting the nanoprobes can be delivered into cells effectively.

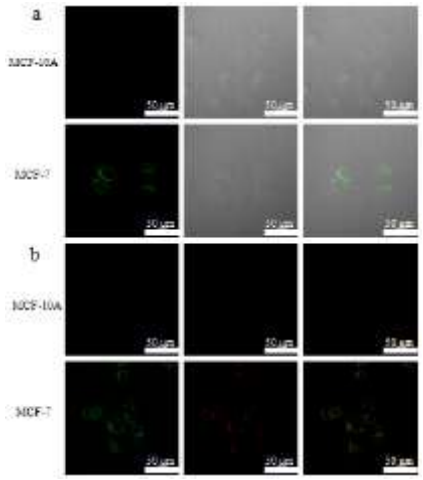


Fig. 3 (a) CLSM images of MCF-10A and MCF-7 cells incubated with the PPy-DNA nanoprobes. (b) Simultaneous detection of multiple mRNAs in living cells. CLSM images of MCF-10A and MCF-7cells incubated with multicolor nanoprobes. Left panels are FAM fluorescence (green, for c-myc mRNA), center panels are Cy3 fluorescence (red, for TK1 mRNA), and right panels are the overlay of FAM fluorescence and Cy3 fluorescence. Scale bar: 50 μm.

Importantly, many cancers are associated with multiple specific mRNAs. The detection of one single type of mRNA might lead to a false positive result. Hence, the simultaneous detection of multiple mRNA targets is of great importance. PPy NPs can act as a common fluorescence quencher toward different kinds of fluorescent dyes. Thus, the proposed method could be expanded easily to detect multiple mRNAs simultaneously. Thymidine kinase 1 (TK1) mRNA was used as a second target, which is a marker for cancer cell proliferation and also highly expressed in MCF-7 cells.[44] The multicolor nanoprobes were used to detect multiple mRNAs simultaneously in living cells. As shown in Fig. 3b, after incubation with the multicolor nanoprobes, both the green fluorescence and red fluorescence were much stronger in the MCF-7 cells than those in the MCF-10A cells. These results indicated the PPy NPs-DNA multicolor nanoprobes are useful for simultaneous detecting multiple mRNAs in living cells.

To explore the applications of PPy NPs in biomedicine, we first tested their potential toxicity on several types of cells. The results revealed that PPy NPs were not cytotoxic to these cells even at high concentration (up to 400 μg /mL), indicating good biocompatibility of the PPy NPs (Fig. S5).

We then studied the photothermal performance of the PPy NPs. An obvious concentration-dependent temperature increase was observed for PPy NPs solutions under laser irradiation (Fig. 4a). The temperature of the PPy NPs solution increased rapidly, while pure water showed a negligible change. The temperature of the solution containing 40 μg/mL PPy NPs solution rose from 25.3 °C to 63.3 °C after 10 min irradiation. It has been reported that cancer cells can be killed after being kept at 42 °C for 15-60 min, and the duration can be shortened to 4-6 min when the temperatures is over 50 °C.[45] These results indicated that the combination of PPy NPs and laser irradiation can kill the cancer cells effectively, and thus PPy NPs could act as an effective NIR photoabsorber for cancer photothermal therapy.

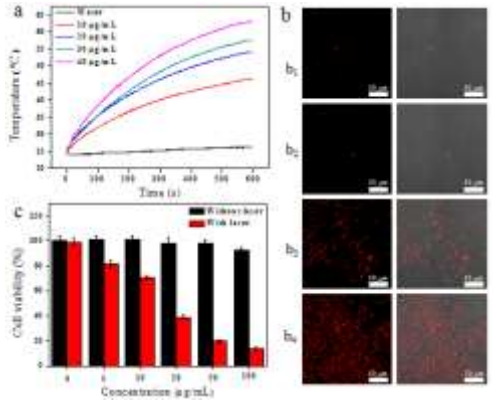


Fig. 4 (a) Temperature elevation over a period of 10 min of exposure to a laser (808 nm, 1W/cm^2) at various PPy NPs concentrations (from bottom to top: 0, 10, 20, 30 and 40 μg/mL). (b) CLSM images of differently treated MCF-7 cells stained with propidium iodide (PI): (b_1) PPy NPs only; (b_2) laser irradiation for 5 min only; (b_3) PPy NPs and laser irradiation for 2 min; and (b_4) PPy NPs and laser irradiation for 5 min. Left panels are PI fluorescence (red, for dead cells), and right panels are the overlay of PI fluorescence and the bright field image. Scale bar: 50 μm. (c) Cell viability of MCF-7 cells exposed to different concentrations of PPy NPs with or without laser irradiation.

Next, we evaluated *in vitro* photothermal ablation capacity of PPy NPs with MCF-7 cells. As shown in Fig. 4b, more cancer cells were dead as the irradiation time was increased. In contrast, with PPy NPs incubation or laser irradiation alone was unable to result in the death of cells. The CCK-8 assays were also performed to quantitatively evaluate the photothermal cytotoxicity of PPy NPs. As shown in Fig. 4c, the cell viability significantly decreased when the MCF-7 cells were treated with PPy NPs and laser irradiation. In contrast, MCF-7 cells treated with the PPy NPs without laser irradiation remained more than 94% viable at the concentration of 100 μg/mL. These results indicated that the PPy NPs could act as an effective NIR photoabsorber for cancer photothermal therapy.

In summary, we have, for the first time, demonstrated PPy NPs-based nanoprobes with low non-specific protein adsorption for intracellular mRNAs detection and cancer photothermal therapy. These nanoprobes present some remarkable features. First, the synthesis of PPy NPs-DNA nanoprobes was easy to perform. Second, PPy NPs can act as a common fluorescence quencher toward different kinds of fluorescent dyes. Thus, the proposed nanoprobes could be expanded easily to detect multiple mRNAs simultaneously. Third, the multicolor nanoprobes display very low non-specific protein adsorption in serum solution, which

makes them promising for mRNAs detection in complex media. Forth, with strong NIR absorption and good photostability, these nanoprobes can serve as an effective agent for cancer photothermal therapy. Collectively, these biocompatible and multifunctional PPy NPs-DNA nanoprobes will provide new opportunities for intracellular mRNAs detection and cancer photothermal therapy, and they will be promising nanoplatforms for developing novel diagnostic and therapeutic techniques.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedures, Table S1 and Fig. S1-S5. See DOI:10.1039/b000000x/

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