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Utilizing Intrinsic Properties of Polyaniline to Detect Nucleic Acid Hybridization through UV-Enhanced Electrostatic Interaction

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Abstract

Detection of specific RNA or DNA molecules by hybridization to “probe” nucleic acids via complementary base-pairing is a powerful method for analysis of biological systems. Here we describe a strategy for transducing hybridization events through modulating intrinsic properties of the electroconductive polymer polyaniline (PANI). When DNA-based probes electrostatically interact with PANI, its fluorescence properties are increased, a phenomenon that can be enhanced by UV irradiation. Hybridization of target nucleic acids results in dissociation of probes causing PANI fluorescence to return to basal levels. By monitoring restoration of base PANI fluorescence as little as 10^{-11} M (10 pM) of target oligonucleotides could be detected within 15 min of hybridization. Detection of complementary oligos was specific, with introduction of a single mismatch failing to form a target–probe duplex that would dissociate from PANI. Furthermore, this approach is robust and is capable of detecting specific RNAs in extracts from animals. This sensor system improves on previously reported strategies by transducing highly specific probe dissociation events through intrinsic properties of a conducting polymer without the need for additional labels.

Graphical Abstract

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INTRODUCTION

Electroconductive polymers (CPs) have many applications in electronic devices and molecular sensors.^{1–7} In biosensors, a promising use of CPs is in the sensing of nucleic acids: a versatile strategy for characterizing cells and their behaviors.^{8,9} Indeed, detection of RNA and DNA is frequently a component of diagnostic technologies used in the clinic and biological research. One of the most common methods of detecting a specific RNA or DNA involves hybridization of probes through complementary base-pairing. This strategy is the basis of venerable southern/northern blotting methods and microarray technology.¹⁰

There are many reports of incorporating different CP chemistries into hybridization-based RNA/DNA sensors that are similar to microarray technology where probes are covalently attached or entrapped in CP matrices.^{11–16} In these formats, electrical and fluorescence properties of CPs have been used to transduce hybridization events, though in many cases secondary detection with electroactive compounds or fluorescent moieties is necessary.^{12,17–23} This strategy has some limitations due to the ability of nucleic acids to spontaneously interact with cationic polyelectrolytes, making it difficult to distinguish bona fide hybridization events from spurious interactions with nontarget DNA or RNA.^{24,25} Furthermore, approaches that use permanent association of probes are frequently reported to be unable to distinguish single base-pair mismatches.¹² This unfortunately makes technologies based on these approaches ill-suited to detect single-nucleotide polymorphisms or distinguish other highly related sequences.

Organic polymers also provide opportunities to generate sensor platforms that are distinct from microarrays. A common alternative takes advantage of the formation of electrostatic complexes between polymers and probes oligos that is driven by interactions between polymer cationic groups and phosphate or nitrogenous bases in DNA.^{26,27} While many polymers have been tested in electrostatic attachment-based sensors, a common observation is that target hybridization disrupts the attachment of probes with polymer causing dissociation.^{28–33} A common strategy to exploit this behavior is to bond a fluorophore-conjugated probe with a polymer that is capable of quenching fluorescence.³⁴ After hybridization, fluorescence is restored due to dissociation of the probe and subsequent dequenching of the label. Studies using this strategy report high specificity, where even a

single base-pair mismatch can be detected. One of the most discriminating sensors used PANI, which gives 60% less restoration of fluorescence if a single mismatch is introduced into target DNA.³²

The goal of this work is to develop a sensor platform that exploits the detachment of probes, but is not exclusively reliant on fluorescence detection. Fortuitously, PANI, which is a proven material for such a sensor, is a versatile CP that has high electrical conductivity, low ionization potential, high electronic affinities, and optical properties that can be used for monitoring biochemical events.^{13,35} It is an excellent candidate for developing a highly specific sensor that can transduce nucleic acid hybridization via a range of physicochemical properties. Here we present a method that uses intrinsic properties of water-dispersed PANI to detect DNA/DNA and DNA/RNA hybridization. Critical to our approach is the use of UV irradiation to enhance interaction between probe oligonucleotides and PANI. This allows interactions of the CP with the probe to be distinguished from interactions with target molecules. The sensor based on this strategy exhibits high sensitivity and specificity for detecting oligonucleotide interactions and is effective for detecting microRNAs (miRNAs) in total RNA extracts from *Drosophila melanogaster* (Fruit Flies). This class of small regulatory RNAs has great potential as biomarkers for numerous diseases. Specifically, the sensor was developed to detect the miRNA species, let-7. This transcript is present in all animals and has a critical, conserved role in tissue homeostasis.³⁶ The capacity of the sensor to detect miRNAs validates its ability to detect specific nucleic acids in complex biological samples.

EXPERIMENTAL SECTION

Materials

Aniline and ammonium peroxydisulfate were purchased from Fisher Scientific, USA. Sodium dodecylbenzenesulfonate (Na-DBS) was purchased from Pfaltz and Bauer, U.S.A. Chloroform was obtained from EMD Chemicals, U.S.A. All the reagents were analytical grade and used without purification. Water used in all measurements and solutions was DNase/RNase-free distilled water. DNA primers were obtained from Eurofins MWG Operon, U.S.A. DNA sequences are the following:

- probe (antisense to let-7) 5'-ACTATACAACCTACTACCTCA-3'
- complementary (identical to let-7) 5'-TGAGGTAGTAGGTTGTATAGT-3'
- mismatch (one base mismatch) 5'-TGAGGTAGTAAGTTGTATAGT-3'
- unrelated oligo, 5'-TAATACGACTCACAGGGAGACCCAAG-'
- long probe, 5'-CATGGTCATAGCTGTTTCCTGACTATACAACCTACTACCTCA-3'
- complementary 2 (M13R) 5'-CAGGAAACAGCTATGACCATG-3'

Phosphate-buffered saline (PBS) was made using preformulated tablets (Fisher Scientific). Urea-polyacrylamide gels were made using the UreaGel system from national diagnostics. Total RNA extraction reagents used the TRI-reagent method (MRC).

Processable PANI Synthesis

Aniline (1 mL, 11 mmol) was completely dissolved in 60 mL of chloroform in a 250 mL round-bottom flask, and the solution was stirred at 600 rpm and cooled to 0 °C. Na-DBS (7.44 g, 21 mmol) was added into the aniline solution and stirred vigorously at 0–5 °C. APS (3.072 g, 13.5 mmol) was dissolved in 20 mL of water in a beaker and added dropwise into the reaction mixture for 30 min to avoid overheating of the reaction mixture. The reaction mixture was stirred at 0–5 °C for 24 h and allowed to reach room temperature for 24 h. The reaction mixture initially turned to milky white, then dark brown, and finally into a dark green colored PANI–DBS dispersion in a chloroform–water mixture. The resultant PANI–DBS solution was filtered in a Buchner funnel and then mixed with 80 mL of chloroform and 120 mL of water in a separation funnel. The solution stood for 24 h, after which the dark green PANI was collected from the separation funnel, while unreacted DBS and APS were left in the aqueous supernatant.

Characterization of Synthesized PANI

The synthesized PANI solution was drop-coated onto a clean 2" × 2" silicon wafer and dried at 40 °C for 48 h in an oven. The PANI-coated silicon wafer was analyzed via FT–IR with a Thermoscientific Nicolet 8700 with g-ATR (Attenuated Total Reflectance) attachment. The sample was analyzed with 128 scans. The PANI solution was diluted 20× with water in which 200 μL of the diluted solution was added into a 96-well polypropylene microplate for UV spectroscopy. The UV absorbance of the PANI solution in the microplate was measured with a Spectramax M3, Molecular Devices, in the range 350–850 nm. The size and distribution of the nanoparticles were measured by dynamic light scattering (DLS) using a Microtrac Nanotrac Ultra NPA150. Particle size and distribution were obtained using a Microtrac Flex software (v.10.6.1), which employs non-negatively constrained least-squares (NNLS) and cumulants analysis to obtain the intensity-weighted “z-average” mean particle size as the first cumulant and the polydispersity index from the second cumulant. High resolution field-emission SEM (FE-SEM) was imaged with Zeiss Sigma variable pressure, field emission gun scanning electron microscope at 10 kV in high vacuum mode with Thermo energy dispersive and wavelength dispersive X-ray detectors (EDS/WDS) at an accelerating voltage of 20 kV. Samples were sputter-coated with silver at an instrument reported thickness of 5 nm.

PANI–Probe Mixing and UV Irradiation

The synthesized PANI had a solids content of 0.12 g/mL. A total of 200 μL of PANI solutions diluted 10× (~2400 μg) or 100× (~240 μg) were mixed with different concentrations of probe ssDNA oligos by gentle rocking for 15 min. Most experiments used 2400 μg of PANI and 0.08 μg of probe. The solution was UV-irradiated at 100 $\mu\text{J}/\text{cm}^2$ for different time intervals. PANI–probe complexes were pelleted by centrifugation at 13300 rpm for 5 min, washed with PBS, and resuspended in water. PANI–probe complex for EDS/WDS measurement was washed with 0.1% SDS.

Emission and Excitation Steady State Fluorescence Measurement

PANI–probe mixtures were added into a 96-well microplate and emission fluorescence was measured in the range 270–850 nm by excitation at 250 nm in a Spectramax M3, Molecular Devices, with an emission peak around 500 nm. The excitation fluorescence intensity was measured by excitation at 350 nm and two peaks were observed for PANI, a high intensity peak at 350 nm and a lower peak at 700 nm. This procedure was also performed for probe-complexed–UV treated PANI.

Hybridization of PANI–Probes with Complementary Oligos and Total RNA Extracted from *Drosophila*

Oligos or total RNA isolated from *Drosophila melanogaster* were added to UV-treated PANI–probe complexes in PBS and incubated at 37 °C for 15 min by gentle rocking. The solution was pelleted by centrifugation at 13300 rpm for 5 min, washed twice with PBS, and resuspended with water. Spectramax M3, Molecular Devices, was used to measure fluorescence.

Fluorescence Microscopy Measurement of Hybridized Duplex

PANI was drop-coated on borosilicate glass coverslip and dried in an oven at 40 °C for 48 h. Probe (8 μg) was added on dried PANI film and irradiated with UV light (100 $\mu\text{J}/\text{cm}^2$) for 1200 ms. The PANI–probe film was washed with PBS and dried at 40 °C for 48 h. Hybridization was subsequently performed for 15 min by adding 8 μg of oligos complementary to the probe coated on PANI. Fluorescent images were obtained using a Leica DMIL at 40 $\times$ magnification, with a 488 nm long pass filter.

Urea–Polyacrylamide Gel Electrophoresis

The fluorescein-tagged probe (8 μg , Fprobe) was mixed and attached via UV with 200 μL (~2400 μg) of PANI. Hybridization was performed as described above, and the PBS wash was collected. Wash solution containing any dissociated oligos (15 μL) was separated by electrophoresis on a urea–polyacrylamide gel. Fprobe fluorescence was visualized with a Bio Rad ChemiDoc MP imaging system.

RESULTS AND DISCUSSION

Synthesis of Processable PANI

The first step in fabrication of a PANI-based sensor of nucleic acids was to generate PANI that was dispersed in water to permit association with probe oligos and targets. To achieve this association, PANI was synthesized by micellar-aided polymer synthesis as previously reported.³⁷ Micelles were generated using a fixed proportion of sodium dodecyl sulfonate (Na-DBS) and ammonium persulfate (APS). The surfactant Na-DBS allows emulsion polymerization of aniline monomers, which facilitates better solubility in a chloroform–water mixture. In micelle-aided polymer synthesis (Figure 1A), the reaction takes place mainly at the micellar–water interface, and during this process, the micelles are in dynamic equilibrium with the surfactant–monomer in the solution.^{38,39} Under such conditions, the micelles present in the solvent not only react but also accelerate the polymerization of

aniline so that PANI will have a relatively high molecular weight while remaining dispersible in the oil–water medium.⁴⁰ The UV absorption spectrum of synthesized PANI–DBS dispersion (Figure 1B) shows a shoulder-like absorption around 350 nm corresponding to π – π^* benzenoid transition and a strong absorption of a polaron band at 750 nm. The latter is an indication of doped PANI in exciton transition.^{41,42} Successful emulsion polymerization of PANI was confirmed by Fourier transform infrared spectral (FT-IR) analysis, which demonstrated a spectral profile as previously described (Figure 1C).⁴³ The peak at 825 cm^{-1} originates from the out-of-plane H deformation of aromatic rings in PANI unit sequences, whereas the peaks at 1500 and 1600 cm^{-1} are due to benzenoid and quinoid moieties of PANI. The characteristic peaks of PANI and DBS were observed at 3450 cm^{-1} corresponding to –NH stretching of PANI, while the peaks at 1060 and 1010 cm^{-1} were attributed to the S=O stretching and –CH stretching of benzenoid rings in the DBS molecule, respectively (Table 1).

Electrostatic Bonds between PANI and DNA Probe Oligos Enhanced by UV

DNA probe oligos were attached to dispersed PANI by electrostatic interaction. These oligos were complementary to let-7, with the ultimate objective being detection of this miRNA. Distinguishing target nucleic acids in a complex biological mixture is a problem for a PANI-based sensor as this CP spontaneously binds to nucleic acids making detection of electrochemical changes caused by target hybridization indistinguishable from nonspecific interaction.^{24,25} To address this, UV irradiation was used to enhance association of probe oligos to PANI. Exposure of polymer surfaces to UV can create polar species and localized charge units increasing wettability.⁴⁴ UV treatment of PANI and probe DNA solution should make the π – π^* bond in PANI more labile, potentially enhancing electrostatic bonds that could form with the negative phosphate groups of probe DNA oligos. Increased bonding will improve differentiation of probes attached to PANI versus spurious association of nucleic acid from a biological sample.

The optimal UV exposure time of PANI–DBS and ssDNA mixture was determined by irradiating at an intensity of $100\text{ }\mu\text{J}/\text{cm}^2$ for various time intervals. Electrostatic attachment of DNA to cationic polythiophene (PTh) has been shown to affect fluorescence of PTh, and this property of PANI was used to monitor the effect of oligo association.⁴⁵ Both the steady state emission and excitation maximum fluorescence intensity show a similar trend as the time of UV exposure of the PANI–DNA complex was adjusted (Figure 2). The higher emission fluorescence intensity at 1200 ms of UV exposure possibly shows electron flux in PANI from phosphate groups of DNA probes (Figure 2A,B). This suggests a high electrochemical adsorption of the negatively charged DNA on positively charged PANI. The increased excitation and emission at 1200 ms indicates that the electron flux from phosphate groups to the delocalized PANI structure increases the benzenoid conjugated state of PANI resulting in higher excitation energy at 350 nm^{11} (Figure 2A,B). At longer exposure times, the fluorescence emission intensity decreases, which could potentially be the result of covalent bond formation between the quinoid moiety of PANI and nitrogenous bases of DNA.⁴⁶

Determining the optimal amount of PANI and probe comprising a sensor is important for establishing a platform suitable for analysis of biological samples. To assess this, different concentrations of probe oligos were tested for their ability to induce changes in PANI fluorescence (Figure 2C). Two concentrations of PANI were tested and both showed that, as the concentration of probe was decreased, there was a concomitant reduction in fluorescence. The threshold for detection of associated oligos was an effect of the mass ratio of PANI to oligo. At the highest concentration of PANI ($\sim 2400 \mu\text{g}$), the lowest amount of oligo that affects fluorescence of the CP was 1 ng. When the lower amount of PANI ($\sim 240 \mu\text{g}$) was used, the lower limit of probe was 0.1 ng. At higher concentrations of probe, the average fluorescence intensity of PANI–probe adduct decreases below base fluorescence intensity. This could be due to covalent bonding between imine groups of PANI and DNA nitrogenous bases, resulting in a loss of fluorescence.^{46,47}

Characterization of PANI–DNA Complexes

Next, the nanostructure of PANI particles was determined before and after association with DNA oligos. Light scattering was used to assess the size of PANI particles present in the chloroform/water emulsion (Figure 3A). A heterogeneous range of particles were observed that spanned approximately 1000 to 100 nm. Association of DNA oligos had a negligible effect on the overall particle population though there was minor skewing to slightly smaller sizes. This may reflect the dynamic nature of the emulsion in which the particles are suspended or effects of exposing PANI to UV. The morphology of PANI was also inspected as a film before (Figure 3B) and after (Figure 3C) DNA immobilization by SEM. Attachment of DNA caused the film to develop a more heterogeneous structure, which like the effect on particle size may be the result of UV irradiation. To verify the association of DNA with PANI, EDS was performed to identify elements associated with PANI (Figure 3D,E). After immobilization of DNA probes with UV, PANI films were extensively washed with SDS, and despite the use of this harsh treatment, phosphorus was observed, indicating that probe oligos were attached.

To better understand the nature of the interaction between PANI and DNA oligos, FTIR was used to identify bonds between different functional groups.

From the spectra a PANI–phosphate bond could be observed at 1270 cm^{-1} which showed elevated interaction after UV exposure (Figure 3F). Sugar phosphodiester bonds and the N–H stretching of purine and pyrimidine bases were also observed at 800 and 1575 cm^{-1} , respectively. This shows probes are electrochemically adsorbed in the PANI structure through electrostatic bonding between phosphate groups in DNA and imine groups PANI and are enhanced by UV exposure (Figure 3G). Importantly, probes associated with this method should be free to base-pair with DNA/RNA as we do not observe the interaction of PANI and probe nitrogenous bases (Figure 3F).

Detection of Hybridization

It was previously reported that hybridization caused electrostatically bonded probes to dissociate from PANI and other cationic polymers.^{28–33} To test if UV-enhanced bonded probes behave in the same way, hybridization was performed with complementary DNA

oligos. Initially, UV spectroscopy was used to determine the polaron (880 nm)/benzenoid (350 nm) ratio of PANI before and after hybridization (Figure 4A). The benzenoid band of the π -conjugated conducting polymers is representative of the electron density of the molecule in a labile state, while the polaron band represents the doped state of the polymer.⁴⁸ The higher electron flux from phosphate groups of attached probes results in lower localization of excited species like excitons leading to lower doped states.⁴⁹ After hybridization of complementary oligos, PANI regained its higher doped state.

Next, changes in the intensity of PANI fluorescence emission was investigated before and after hybridization. Similar to what was observed with UV spectroscopy, hybridization of complementary oligos reduced PANI fluorescence caused by association of probes (Figure 4B). This effect was highly specific to the sequence of target oligo added during hybridization. Introduction of a single mismatch into the oligo significantly impaired restoration of PANI base-fluorescence relative to when hybridization was performed with a complementary oligo. Similar to other platforms that use electrostatic attachment of probes, this indicates that a PANI-based sensor with UV-immobilized probes will be highly specific to target nucleic acids.^{28–33}

Next, PANI coated on borosilicate glass was tested as a format for the sensor platform (Figure 4C). Fluorescence of the PANI film was observed using conventional fluorescent microscopy. After coating, probes were electrostatically adsorbed to PANI and irradiated by UV that, as was seen in dispersed PANI, increased fluorescence. Hybridization was then carried out with complementary oligos. Also similar to what was observed with PANI dispersed in water, a dramatic loss of fluorescence was observed upon hybridization. Together, these results demonstrate that PANI can be utilized to detect nucleic hybridization without the need for secondary detection or covalent probe attachment.

Hybridization Causes Dissociation of Probes from PANI

The results of hybridization experiments with UV immobilized probes were consistent with the previously described dissociation from CPs after hybridization.^{28–33} To confirm dissociation, the presence of dissociated probe was monitored in hybridization solution (Figure 5A). A probe labeled with 6-fluorescein amidite (6-FAM) was attached to PANI by UV, and subjected to hybridization. After pelleting of emulsion PANI by centrifugation, the supernatant was separated by electrophoresis on a 8 M urea denaturing polyacrylamide gel (Figure 5A). In the absence of target oligo, very little Fprobe was found in the supernatant. However, when complementary oligo was added, a large amount of Fprobe was observed in the supernatant, indicative of probe dissociation. In contrast, minimal probe dissociation was observed when hybridization was carried out with oligos with an unrelated sequence or oligos containing a single mismatch. Similar to the nonhybridized condition, the addition of unrelated oligo did not result in any appreciable increase in dissociation of Fprobe. Hybridization with the single mismatch oligo did result in some dissociation of probe, but was dramatically less than when using perfectly complementary oligos. These results reconfirm detachment of UV immobilized probes occurs after hybridization (Figure 5C). If electrostatic interaction between phosphate groups of DNA and PANI is a major driver of association, it would appear that dissociation may be caused by the formation of the helical

structure of double-stranded DNA leading to conformational changes that break the electrostatic interaction. Furthermore, the ability of a single mismatch to inhibit dissociation highlights the stringency for formation of perfectly helical DNA devoid of bulges due to unpaired bases.

To further test the versatility of this interaction, we investigated the ability of the system to detect the nucleic acids of greater length (Figure 5B). A probe was immobilized on PANI that was twice as long as the one used in previous experiments (long probe). A total of 21 nucleotides of the oligo were identical to probes used previously (complementary to let-7); the remaining residues were of unrelated sequence (complementary to the common PCR primer, M13R; Figure 5B). After hybridization with complementary oligo1, a modest decrease in the fluorescence was observed. This indicated that the unpaired residues remained attached and that the sensor will not detect fragments of nucleic acid that do not span the length of the probe. However, when complementary oligo 2 (M13R) was added, dissociation of the probe occurred. This demonstrated that probes could be designed to detect longer nucleic acids and that the ability of the probes to dissociate is not necessarily limited to a particular oligo length (Figure 2C).

Sensitivity and Specificity of PANI Sensor

Finally, experiments were performed to assess the sensitivity of the PANI-based sensor and its usefulness when used with biological samples. The detection range was determined by hybridizing a series of complementary oligo concentrations to PANI–probe complexes (Figure 6A). The fluorescence intensity of PANI was then measured to evaluate the ability of the different amounts of complementary oligo to restore base PANI fluorescence. When complementary oligo was added in molar excess of attached probes, higher fluorescence was observed possibly due to direct interaction of oligo with PANI. Equimolar amounts of complement completely restored PANI fluorescence. At other concentrations, there was a clear trend showing decreasing amounts of complement oligo, resulting in less reversion of PANI fluorescence. Ultimately, the sensor is able to detect a complementary oligo that is present at a concentration around 10 pM. Optimization of PANI and probe concentrations will likely yield even greater sensitivity. Cogent to this point, multiple concentrations of PANI and probe could be used to construct a viable sensor (Figure 2C). Determining the most efficacious amount of PANI and probes in a biological sensor will be the focus of future work. However, despite the nonoptimized nature of the sensor system presented here, the detection limit was lower than other probe-dissociation-based sensors or those that do not use secondary detection.^{12,28–33}

The performance of the sensor was tested with a biological sample by detecting a specific transcript in total RNA extracted from *Drosophila melanogaster* (fruit flies). Throughout this study, the probes attached to PANI were designed to be complementary to the miRNA let-7. This miRNA is not consistently expressed across the stages of the fruit fly life cycle. It is present in adult flies, but not early stages such as larva. Total RNA was extracted from adult and larval flies, and 1 μ g from each sample was hybridized to PANI–probe complexes (Figure 6B). Consistent with expression of let-7 in adult flies, we observed a strong reduction of PANI fluorescence after hybridization. This reduction was not seen when

hybridizing PANI–probes with RNA extracted from larva, which do not express let-7. This result indicates that the platform is sufficiently robust to detect the presence of a specific nucleic acid, even when confronted with the complex mixture of nucleic acids comprising biological samples.

CONCLUSIONS

In this research, a sensor of nucleic acids was developed based on PANI and UV-immobilized probes. PANI was synthesized by micelle-aided polymerization with sodium dodecyl benzenesulfonate, resulting in water dispersible PANI that was able to interact with nucleic acids in solution. A strategy was developed for electrostatic immobilization of probes oligos on PANI that used enhancement by UV irradiation. Increased binding of PANI to probes from UV exposure significantly altered the fluorescence of PANI. This exposure allowed for the attachment of probes without the formation of covalent bonds, while utilizing properties of PANI in signal detection. Furthermore, this platform was capable of detecting hybridization of oligos complementary to probes with great specificity, distinguishing between perfectly complementary oligos and ones only having a single mismatch. The platform also demonstrated high sensitivity and was capable of detecting amounts of target oligo lower than other published methods that used probe-dissociation-based transduction or detection without secondary compounds.^{12,28–33} The sensor was also robust enough to detect expression of small RNAs in total RNA extract. Finally, we show that the sensor relies on dissociation of probes via duplex formation to transduce hybridization. This configuration of the sensor provides several improvements on previous strategies. A label is not used, reducing overall cost of fabrication, minimizes the complexity of the analysis, and excludes the need for fluorescent probes, which might affect the binding interactions.⁵⁰ This technology could be highly versatile with different oligo probes attached in a microarray-like format to detect many miRNAs simultaneously. Multiplex detection of gene expression could also be achieved through using polymers that have different properties such as nonoverlapping fluorescence emission spectra. The use of this strategy might also be suitable for use with DNA-based aptamer systems to expand beyond sensing nucleic acids with UV attached probes.⁵¹ Finally, other reports describing sensors based on probe dissociation rely on fluorescent labels, and could not take advantage of all CP properties to transduce hybridization. Future work will be aimed at exploiting other properties of PANI, such as electrochemical methods, to detect nucleic acids. Such sensors will likely be compatible with innovative systems such as creating sensor arrays from hydrogels or other functional nanomaterials.^{52,53}

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Figure 1. Synthesis of processable PANI–DBS. (A) Generation of PANI via micellar-aided polymer synthesis. Aniline and Na–DBS are combined to yield micelles. Addition of APS triggers PANI synthesis. (B) UV spectra of synthesized PANI–DBS. (C) FTIR spectra of synthesized PANI–DBS.

Figure 2.

Optimizing UV exposure to promote formation of PANI–DNA probe complexes. (A, B) Solutions were exposed to UV at $100 \mu\text{J}/\text{cm}^2$ for durations indicated on the y-axis. Fluorescence (A) and excitation (B) of PANI were measured to assess the effects of UV on the interaction of PANI and DNA. (C) Fluorescence emission of two amounts of PANI before and after UV-mediated binding to probe oligos complementary to let-7.

Figure 3.

Molecular interactions between DNA and PANI. (A) Light scattering showing the range of sizes of PANI particles before (red line) and after DNA immobilization (blue line). (B) SEM imaging showing PANI film morphology. (C) SEM of PANI film morphology after UV exposure. (D, E) EDS analysis of PANI film before (D) and after DNA attachment (E). Elements recovered indicated above the appropriate signal peak. Mild UV exposure enhances the interaction between the phosphate groups in ssDNA and PANI. (F) FTIR spectra of PANI, PANI mixed with DNA probe, and after UV exposure of PANI–DNA probe mixture at 100 $\mu\text{J}/\text{cm}^2$ for 1200 ms. (G) Stronger electrostatic bond by UV irradiation of PANI–DNA probe adduct.

Figure 4.

Hybridization of oligos complementary to UV-immobilized probes reverses elevated PANI fluorescence. (A) Polaron/benzenoid ratio of PANI, PANI with attached probe (PANI + probe) and after hybridization with a complementary oligo (PANI + probe + comp). (B) Changes in PANI fluorescence at 500 nm before and after hybridization. Measurements were made before probe attachment (PANI), after probe attachment (PANI + probe), after hybridization with oligos with one mismatch (PANI + probe + 1 mis oligo), or hybridization with a 100% complementary oligo (PANI + probe + comp). (C) Fluorescence microscopy showing images of PANI coated on borosilicate glass (PANI), PANI with attached probe (PANI + probe), and after hybridization with 100% complementary oligo.

Figure 5.

Hybridization of immobilized probes results in detachment from PANI. (A) Imaging of FAM conjugated let-7 oligo probe (Fprobe) after urea-PAGE electrophoresis. Fprobe was immobilized on PANI and mixed with hybridization buffer (PBS). After a 15 min incubation, PANI was removed by centrifugation, and the supernatant loaded on a urea-PAGE gel. In lane 1, no oligo was added. In lane 2, a DNA oligo complementary to Fprobe was added. In lane 3, an oligo of unrelated sequence was added. In lane 4, an oligo was added that had a single mismatch to the Fprobe. (B) Consequences on PANI fluorescence when a longer probe is used. Fluorescence was compared after no oligo was added, an oligo was added that was complementary to half the probe (comp), and when two oligos (comp1 + comp2) were added that together span the length of the probe. (C) Diagram showing detachment of probes from PANI after duplex formation with complementary target nucleic acid. Three conditions are compared: use of a short probe with complementary oligo, use of a long probe with an oligo that is partially complementary, and use of a long probe where two oligos are used to pair with all the bases of the long probe.

Figure 6. Sensitivity and specificity of PANI-based nucleic acid hybridization sensor. (A) PANI fluorescence after hybridizing increasing concentrations of complementary oligo. (B) Detection of let-7 miRNA in total RNA isolated from adult (let-7 expressing), but not in larval (no let-7 expressed), fruit flies.

Table 1

FTIR Characteristic Peaks of PANI–DBS

characteristic bands	peak position (cm ⁻¹)
out of plane H deformation of aromatic rings	825
C–H stretching of benzenoid rings of DBS	1010
S=O stretching	1060
C–C stretching	1170
benzenoid stretching	1500
quinoid stretching	1600
aliphatic C–H stretching	2950
N–H stretching	3450