A Perspective on Polythiophenes as Conformation Dependent Optical Reporters for Label-Free Bioanalytics

Gaurav Sinsinbar, Alagappan Palaniappan, Umit Hakan Yildiz, and Bo Liedberg*

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Poly(3-alkylthiophene) (PT)-based conjugated poly-electrolytes (CPEs) constitute an important class of responsive polymers with excellent optical properties. The electrostatic interactions between PTs and target analytes trigger complexation and concomitant conformational changes of the PT backbones that produce distinct optical responses. These conformation-induced optical responses of the PTs enable them to be utilized as reporters for detection of various analytes by employing simple UV−vis spectrophotometry or the naked eye. Numerous PTs with unique pendant groups have been synthesized to tailor their interactions with analytes such as nucleotides, ions, surfactants, proteins, and bacterial and viral pathogens. In this perspective, we discuss PT-target analyte complexation for bioanalytical applications and

highlight recent advancements in point-of-care and field deployable assays. Subsequently, we highlight a few areas of critical importance for future applications of PTs as reporters, including (i) design and synthesis of specific PTs to advance the understanding of the mechanisms of interaction with target analytes, (ii) using arrays of PTs and linear discriminant analysis for selective and specific detection of target analytes, (iii) translation of conventional homogeneous solution-based assays into heterogeneous membrane-based assay formats, and finally (iv) the potential of using PT as an alternative to conjugated polymer nanoparticles and dots in bioimaging.

KEYWORDS: *Polythiophenes, Conjugated polyelectrolyte, Fluorescent reporters, Bioimaging, Optical biosensors, Point-of-care testing, Nucleotides, Proteins, Ions, Bacterial pathogens*
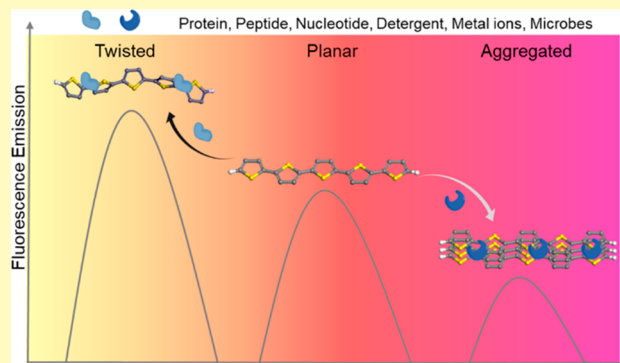
Conjugated polyelectrolytes have been of great interest to scientists because of their attractive optical properties.[1] Polythiophene-based polyelectrolytes constitute a class of CPE that have been explored extensively for bioanalytical applications. In particular, PT-based CPEs are preferred because of their (i) affinity-responsive optical and electrical properties, (ii) enhanced optical responsiveness compared to synthetic dyes, and (iii) low photobleaching. PTs have been synthesized using various electrochemical and metal-catalyzed polycondensation polymerization strategies.[2−4] Ferric chloride, palladium, and vanadium catalyzed oxidative polymerization of thiophene monomers also have been explored for the synthesis of PTs.[5−7] PTs are based on 2,5-polymerization of thiophene monomers (Figure 1A,B).[8−10] PTs with different side chains and functional groups R also have been synthesized to tailor their interaction with target analytes for developing optical reporters for the detection of analytes such as small molecules,[11−15] proteins,[16−22] peptides,[23−25] nucleic acids,[26−29] bacterial pathogens,[30−33] polysaccharides,[34] and volatile organic compounds.[35−38] Garnier et al. presented the first evidence of the presence of crystalline structure of PT with a hexagonal lattice.[39] PTs contain delocalized electrons along

the conjugated polymeric backbone that determine their optical and electrical properties (Figure 1C). The conformations of PT backbones and the density of their side chains affect the delocalization of electrons along the backbone, which modulates their optical and electrical properties. These conformations can be planar, partly twisted, or helical, which affects the chirality of PTs, the effective conjugation length, and the delocalization of electrons along the chain (Figure 1D−G).[21,23,24,39−42]
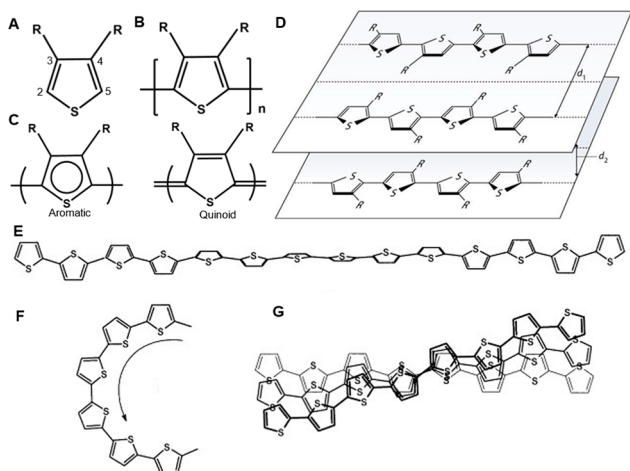
The precise nature of these conformations has been a matter of significant research interest. PTs in planar conformation allow for facile electron migration due to an increased conjugation length that red shifts in the emission spectrum. In contrast, a twisted backbone hinders the migration of

Figure 1. Chemical structures of (A) thiophene, (B) polythiophene, and (C) resonance structures of PT. (D) Illustration of π stacking of planar PT chains. The d_1 and d_2 distances depend on the side chain groups R. Reprinted with permission from ref 40. Copyright 2010 Springer Nature. Illustration of helical organization of PTs: (E) helical transoid (larger pitch). (F) Helical cisoid (smaller pitch). (G) Helical packing of predominantly planar chains. Reprinted with permission from ref 47. Copyright 2000 Elsevier.

electrons resulting in a decreased conjugation length and a blue shift of the emission spectrum. Thus, changes in the conjugation length directly affect the band gap of PTs, which eventually causes significant changes in absorption and emission. Moreover, stacking of PT chains results in aggregation, causing abrupt changes in optical response; a red shift in the emission spectrum and a decrease in fluorescence intensity due to the nonradiative energy transfer.[20−23,29,41,43−46] The collective response of PTs in the form of backbone conformational rearrangement or aggregation is the fundamental sensing mechanism of PTs, making them highly sensitive and responsive to their surroundings.

Based on theoretical calculations, Cui and Kertesz suggested that methyl-substituted PTs preferentially adopt helical conformation. They further showed that transoid and cisoid helices only display marginal energy differences (Figure 1 E,F).[42] The backbone conformation of PTs can be altered by solvatochromic, thermochromic, and affinity-chromic effects. Employing the PTs with unique side chains R, Figure 1A, that specifically interact with a target and bring about a conformational change in its backbone offers an attractive avenue for developing biosensors to detect various analytes. The interactions between side chains and the analyte induce conformational changes in the PT backbone or a change in the aggregation state of PTs in solution and in solid-state, further modulating the optical spectra.[11,14,23,24,27,43,48−50]

Even with high sensitivity and quantum yield, easy tunability, and various other advantages of using PTs as reporters in optical sensors, there are also several limitations in their applications. A significant drawback is the variation in the polymerization step of thiophene monomers, which often results in a polydisperse material. Moreover, the synthesis process introduces batch-to-batch variations and the characterization of these materials is also cumbersome. Growing research attention is focused on developing better synthesis approaches to circumvent these issues. In this context, we elaborate on recent advancements in utilizing PTs as reporters for point-of-care and field-deployable assays. The perspective

further discusses several areas of critical importance for the development of improved PT-based reporters in bioanalytics. We also discuss the potential of using PT and related conjugated materials and dots for bioimaging.

THEORY OF CPE COMPLEXATION WITH ANALYTES

The theory of CPE complexation with analytes has been extensively discussed over the last two decades. The complexation phenomena have been described as an associative phase separation of the polyelectrolyte chains followed by the formation of dense polymer-rich phases known as polyelectrolyte complexes.[51] The early works that describe the theory of liquid−liquid phase separation leading to complex coacervation were systematically conducted by Voorn and Overbeek,[52] and the coacervation in aqueous mixtures of gum arabic and gelatin, both naturally occurring polyampholytes, were the basis of the mean-field theory developed by Voorn and Overbeek.[53] The estimation of the free energy of the system relied on the entropy of mixing, with Flory−Huggins expression,[54] and a Debye−Hückel term to include electrostatic interactions. The Voorn and Overbeek theory (VO) was valuable to explain coacervation phenomena; however, it did not explain the complete physical description of complexation of polyelectrolytes in solutions. Therefore, the random phase approximation (RPA) was suggested as a realistic approach to incorporate finite size and connectivity of charges into electrostatic free energy description, which is a combination of the free energy of the neutral reference solution with short-range interactions and a free energy contribution from the long-range electrostatic interactions. The RPA assisted in accounting for excluded volume effects and non-zero size of the ions in complexation. However, it lead to profound disagreements with the VO model predictions.[55−61]

The RPA provided a deeper understanding of the phase separation in solutions of oppositely charged polyelectrolytes. However, it did not provide an accurate description for systems with high charge densities, owing to overestimated charge correlations. Fredrickson and co-workers alternatively employed field-theoretic simulations to solve the full field theory and to overcome the limitations associated with the assumptions of weak charge and density correlations.[62−64] Other studies have introduced simulations of polyelectrolyte complexation at the molecular and macromolecular levels.[65−68] The major issues in complexation simulations appeared due to flexibility of polyions and added salt that affects the resultant shape and size of the complexes. In a recent study,[15] poly(L-lysine) (PLL) polycation and short segment double-stranded DNA polyion complexation was elaborated on in the presence and absence of low molecular weight salt. Figure 2A shows the clusters formed by the complexation of long polycations with short polyanions as a function of salt concentration at a charge ratio of 0.99 for (i) flexible−flexible and (ii) rigid−flexible polyions. The profound effect of added salt appeared as dissolution of polyelectrolyte complexes with increasing concentration. It also was shown that rigid−flexible complexes release many more polyions by increasing salt concentration due to limited conformational flexibility. More specific simulations for polythiophene and DNA complexation were presented by Surin and co-workers as shown in Figure 2B and C.[29,69] Figure 2B shows single-stranded (ss) polyA-PT and polyT-PT complexation. Analysis revealed that electrostatic interactions played a pivotal role in PT-DNA complexation. In
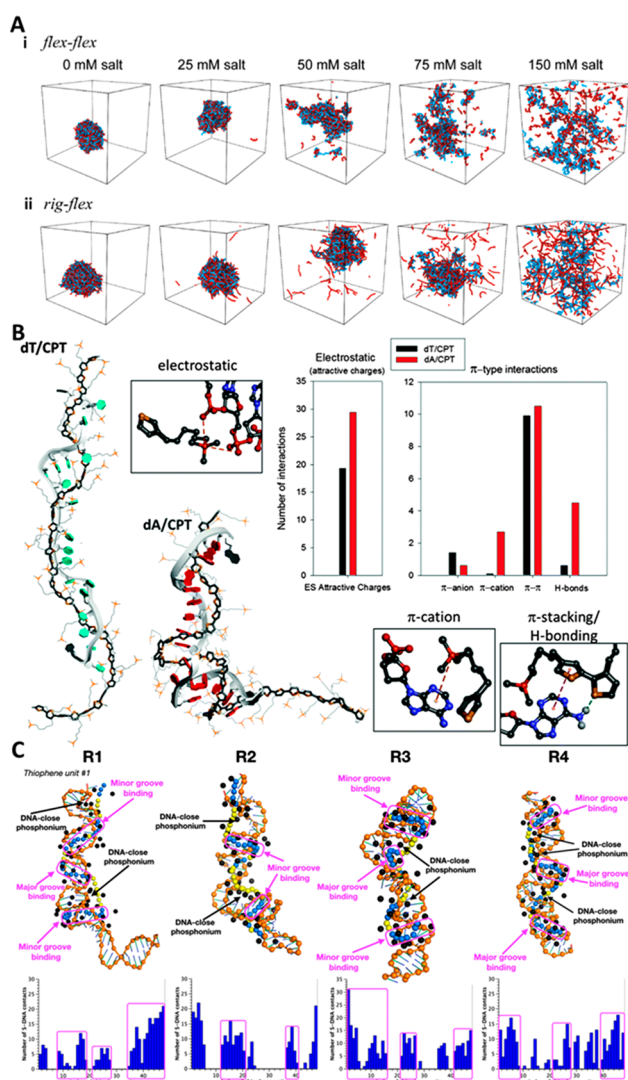
Figure 2. (A) Simulations showing complexation of asymmetric oppositely charged polymers as a function of polymer concentration, forming (i) flexible−flexible complexes and (ii) rigid−flexible complexes. Reprinted with permission from ref 84. Copyright 2020 American Chemical Society. (B) Illustration of dT/cPT and dA/cPT complexes extracted at the end of the molecular dynamics (MD) simulations and possible intermolecular interactions are represented in the boxes. The bars represent the average number of intermolecular interactions for the two complexes based on MD simulations. Reprinted with permission from ref 29. Copyright 2020 Royal Society Of Chemistry. (C) Illustration showing thiophene moieties that interact with the DNA double helix on final MD snapshots of dsDNA-polythiophene complexes. Reprinted with permission from ref 69. Copyright 2020 John Wiley and Sons.

addition, $\pi-\pi$ interactions between thiophene rings and nucleotide bases also were found to affect the size and shape of the resultant complexes.[15] More importantly, for homonucleotide sequences (polyT or polyA), the complexation between ssDNA and cationic PT (cPT) was found to be a right-handed conformation of the polymer chain (referred to as induced circular dichroism (ICD) signatures in the CD spectrum). However, cPT complexation with oligonucleotides of a mixed sequence (in ssDNA or double stranded dsDNA topologies) yielded left-handed ICD signatures.[70,71] Later, multiple binding modes of DNA-cPT were studied in an another work, where cPT moieties exhibited binding on minor

grooves and nonbinding motifs.[29] In Figure 2B, multiple binding modes of the cPT and dsDNA are illustrated for the replicas. The number of sulfur atom−DNA interaction histograms for the different replicas reveal that the initial conformation of dsDNA limit or intensify the cPT−DNA interactions. As explained in the same study, the polymer can approach DNA in several ways, leading to multiple possibilities of "wrapping" around DNA. Moreover, the polymer can adopt many types of conformations owing to its intrinsic flexibility as compared to DNA, whereas the DNA backbone is more conserved.

The optical properties of PTs are dependent on aggregation of the polyelectrolyte chains, regioregularity, and backbone conformations. These factors are significantly affected by the interaction of PTs side chains with target molecules. Although these interactions have been made highly specific with the use of biotin−avidin[72] and antigen−antibody,[73] for most PTs, the interactions are not highly specific, but are governed by hydrogen bonds, nonpolar and electrostatic interactions between the PTs side chains and target molecules. Ultimately, the interactions between the side chain and target molecule bring about the conformational change in the backbone of PTs from planar to twisted or vice versa and can even induce aggregation of the PT chains. Twisted to planar transition makes PTs an ideal conformation-dependent optical reporter in label-free biosensing of polynucleic acids,[74] proteins, proteins,[18] enzymes,[75−79] carbohydrates,[80] small molecules,[11−15] and volatile organic compounds.[35−38] Elaborate discussions have been presented in the relevant literature concerning conformation induced optical transitions that are attributed to the band gap alteration between HOMO and LUMO of PTs upon binding event or engineered by creating disorder[81] or doping using side chains.[82,83]

APPLICATIONS OF PT REPORTERS

Easily tunable optical and electrical properties, enhanced responsiveness, and resistance to photobleaching are a few major advantages of PTs over other reporter molecules. These properties have inspired extensive use of PTs as a reporter molecule in optical sensors. The net charge, spacer length and type, and functional group of the side chains at the 3- and 4-positions of the thiophene rings easily can be modified to optimize interaction with analyte molecules.[85] PTs can be polar/apolar, cationic/anionic, or zwitterionic depending on the charge(s) and functional groups present on the side chain. Monomeric thiophenes bearing different side chains have been mixed or copolymerized to display entirely novel properties and unique responses upon interactions with analyte molecules. The side chains with various functional groups and charge at the 3- and 4-positions of thiophene rings not only make the hydrophobic backbone soluble in polar solvents, but are also directly involved in both electrostatic and hydrophobic interactions with different analyte molecules.[86−88]

Conjugated polyelectrolytes display distinct color transitions between deep violet ($\lambda_{max} \approx 550$ nm) to bright yellow ($\lambda_{max} \approx 400$ nm) as a result of planar-to-nonplanar (from highly conjugated to less conjugated) conformational changes of the PT backbone (Figure 1D−G). The conjugated (planar) state of the backbone is susceptible to intermolecular and intramolecular (chain folding) interactions or aggregation as a result of the interactions with analyte molecules, thereby producing an optical response in the form of changes in the absorbance and fluorescence spectra. More importantly, the

forces which define these interactions and backbone conformation changes of PTs are hydrogen bonds, nonpolar and electrostatic interactions, which potentially may lead to aggregation, gelation, or crystallization.[89,90] Chiral PTs show optical activity in the $\pi-\pi^*$ transition region. However, optically inactive nonchiral PTs become chiral and show ICD upon interaction with chiral molecules.[91−94] For example, the formation of a one-handed helical structure because of the acid−base complex formation between PTs and chiral molecules may introduce chirality in optically inactive PTs.[91−94] The tunability and processability of PTs imposed by the richness in chemical flexibility of the side chains have paved the way for a plethora of analytical and device applications. Specifically, PT's optical properties have attracted considerable interest for developing colorimetric sensing concepts (e.g., for detection of nucleic acid,[26−29,74] ions, proteins,[18] polysaccharides,[34,80] small molecules,[11−15] and pathogens[30−33]) and for cell targeting and imaging.[95,96] In addtion, PTs also have been explored for various other applications as gene delivery vehicles,[97] anticancer,[98] anti-fungal, or antibacterial agents,[99−101] molecular switches/wires, and photovoltaic devices.[102−106] This perspective is devoted to the optical properties of PTs for the development of label-free bioanalytical tools, and potentially also for cellular imaging strategies.[107−112]

Optical Biosensors for Detection of Ions, Small Molecules, and Surfactants.
Polythiophenes have been widely used for the detection of small molecules, e.g., metal ions, halides, surfactants, and deoxynucleoside tri/di/mono-phosphates. The PTs and oppositely charged species undergo electrostatic interactions to bring about the optical changes, which makes them crucial for developing optical assays for biosensing applications.

Metal Ions. Metal ion contaminants in water and food cause severe health-related problems in humans. Point-of-care sensors for faster and cheaper monitoring of various metal ions, including manganese and lead, both of which are powerful neurotoxins, have been developed. Chen et al. developed a sensitive and selective label-free DNAzyme-based sensor for lead (Pb^{2+}) ions by using nucleotides and cPTs.[50] They used a Pb^{2+} specific DNAzyme, which is activated upon binding with Pb^{2+}. In the absence of Pb^{2+} ions, the dsDNA of DNAzyme interacts with PT to form a ternary complex with high fluorescence (Figure 3B). However, in the presence of Pb^{2+} ions, the DNAzyme cleaved its substrate producing ssDNA. The ssDNA interacts with PT forming a duplex with a low fluorescence signal.[50]

Colorimetric aluminum ion (Al^{3+}) detection has been suggested by Tu et al.[113] They employed Al^{3+}, specific aptamers, and PT, that enabled selective detection of Al^{3+} in drinking water. Tu et al. suggested the use of copolymers instead of single monomer-based PTs (Figure 4A). The PT copolymer formed a complex with ATP resulting in quenching of the fluorescence of PT copolymer and a significant color change. However, with the addition of Al^{3+} ions, the complex is disassociated, resulting in the recovery of fluorescence and color. Thus, as shown in Figure 4B, the assay is based on the fluorescence and color recovery of a PTs-ATP complex in the presence of Al^{3+} ions.[113]

Ho et al. demonstrated the selective detection of iodide (I^-) ions using poly(3-alkoxy-4-methyl thiophene).[114] Their approach was based on electrostatic interaction (self-assembly of two opposite charges) and the conformational change of the
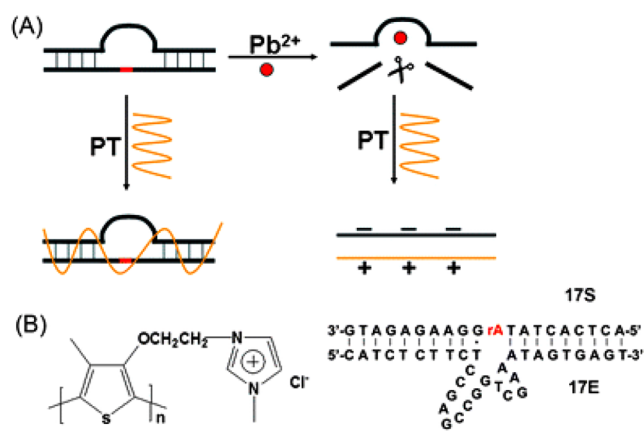
Figure 3. (A) Schematic illustration of label-free fluorescent detection of Pb^{2+} ions. The addition of Pb^{2+} induced cleavage of the dsDNA into ssDNA, resulting in electrostatic interactions between ssDNA and PT, and leading to planar conformational variations with a decreased fluorescence signal. (B) Chemical structure of the cPT and secondary structure of the 17E DNAzyme. Reprinted with permission from ref 50. Copyright 2012 Royal Society Of Chemistry.
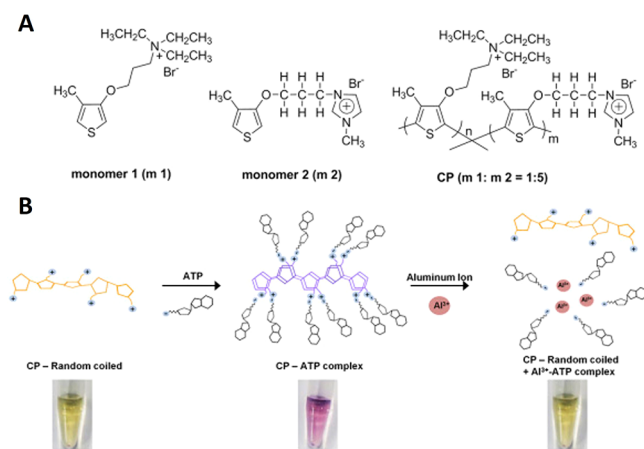

Figure 4. (A) Structure of monomer 1, monomer 2, and its copolymer PT. The ratio of the monomers was varied to optimize the optical response. (B) Illustration of the principle of Al^{3+} detection using copolymer PT-ATP complex. Reprinted with permission from ref 113. Copyright 2016 Elsevier.

cPTs.[114] Their findings suggested that the I^--induced optical properties were strongly dependent on the nature and the length of the side chain of cPTs. Wang et al. further investigated the temperature-dependent compaction/decompaction transition process of PTs in the presence of KI and showed the potential use of PTs as a temperature indicator (Figure 5).[115] Wang et al. reported the use of the cross-linked PTs nanostructure for selective detection of cupric (Cu^{2+}) ions.[116] The Cu^{2+} ion selectively binds to the water-soluble conjugated polymer nanostructures resulting in a decreased fluorescence due to photoinduced electron transfer.[116]

Ghosh et al. reported the specific fluorometric detection of cyanide (CN^-) ion using PTs (Figure 6).[117] Poly(N,N-dimethylaminoethyl methacrylate) (PTDMA) with iodide counterion (CPT-I) was used in this assay. The CPT-I showed lower fluorescence intensity than that of PTDMA because of excitonic energy transfer from the CPT backbone to quencher I^- ions. CN^- ions did not show any quenching property, but had a higher preference to interact with PTDMA
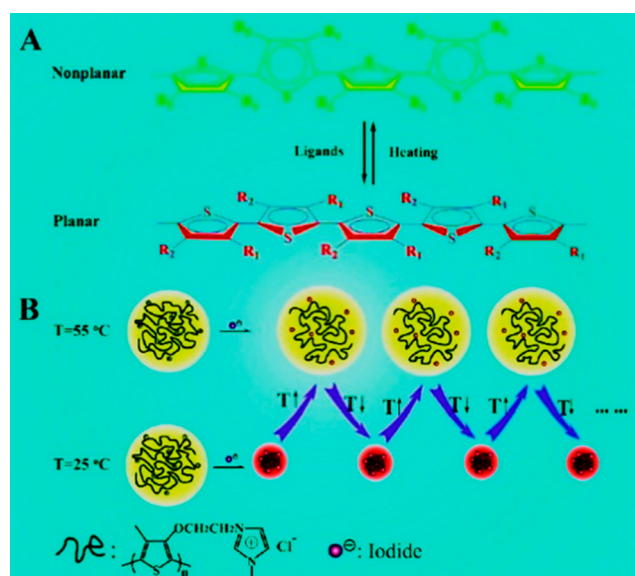
Figure 5. Illustration showing (A) the conformational changes in PTs when exposed to ligands or varying surroundings and (B) compaction–decompaction of PTs in the presence of KI at different temperatures.[115] Reprinted with permission. Copyright 2011 American Chemical Society.
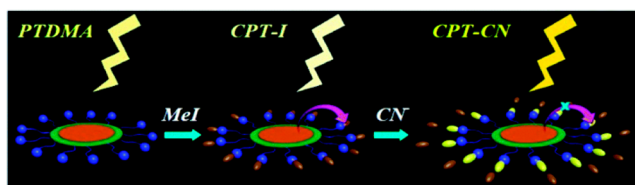

Figure 6. Illustration showing enhancement of fluorescence intensity in the presence of CN^- ion. The orange disk represents the PT chain, the green shell stands for a methacrylate chain, a blue sphere with a blue chain represents $-NMe_2$ groups, the brown capsule represents I^- ion, and the yellow capsule represents the CN^- ion. Reprinted with permission from ref 117. Copyright 2015 Royal Society Of Chemistry.

as compared to I^- anions. Thus, the CN^- ions substituted the I^- anions and resulted in an enormous increase in the fluorescence proportional to their concentration.

Phosphates in Nucleotides. Nucleotide phosphates are critical for various biological reactions and metabolic processes. The intracellular deoxynucleoside triphosphate (dNTP) pool is essential for genomic stability and cancer development.[118] The purines adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine are important extracellular signaling agents.[12] This turns nucleotide phosphates into a critical class of biomarkers to detect using easy-to-use optical assays.

Yao et al. used the poly(3-alkoxy-4-methyl thiophene) derivative poly(3-(4-methyl-3′-thienyloxy) propyltrimethylam-monium (PMTPA) for developing an optical sensor to detect and distinguish 15 nucleotides along with monophosphate and pyrophosphate with 100% confidence limits.[27] Their findings suggested that the multiple negative charges (triphosphates compared to monophosphates) and more hydrophobic nucleobase moieties (purines relative to pyrimidines) are crucial for determining the interactions between PMTPA and 15 nucleotides (containing phosphates: XNPs, where, X = A, U, T, G, C, and N = mono, di, tri, Figure 7A). The
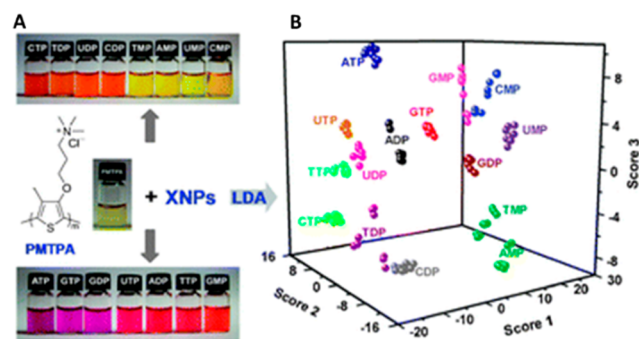
Figure 7. (A) Colorimetric response of PMTPA solutions induced by the addition of different nucleotide phosphates (XNPs). (B) Three-dimensional LDA score plot for the analysis of the effect caused by the addition of 15 nucleotides to PMTPA. Reprinted with permission from ref 27. Copyright 2009 Royal Society Of Chemistry.

electrostatic, hydrophilic, and hydrophobic interactions cause planarization of the PMTPA backbone and formation of PMTPA aggregates with different molecular ordering, which produce distinct optical responses. The optical responses were analyzed using linear discriminant analysis (LDA) (Figure 7B).

Tang et al. reported the interactions of cPT (poly[3-(3′-N,N,N-trimethylamino-1′-propyloxy)-4-methyl-2,5-thiophene hydrochloride], cPT) and ATP to detect and monitor glucose phosphorylation by hexokinase in real-time.[79] Glucose is catalytically phosphorylated to D-glucose-6-phosphate (G-6-P) by hexokinase in the presence of ATP and magnesium (Mg^{2+}) ions (Figure 8). During this process, ATP is converted into
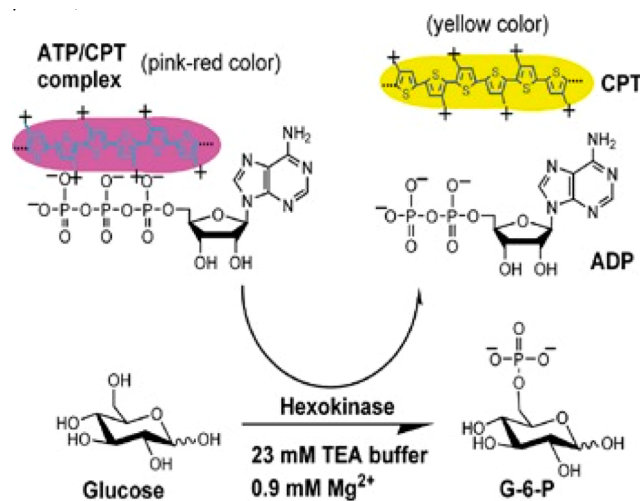
Figure 8. Illustration representing a colorimetric assay for detection of glucose based on hexokinase catalyzed ATP-dependent glucose phosphorylation using cPT. Reprinted with permission from ref 79. Copyright 2008 John Wiley and Sons.

ADP. ATP and ADP interact with cPT in different ways, yielding pink-red and yellow color, respectively. Yildiz et al. reported the use of poly[1-(3-((4-methylthiophen-3-yl)oxy)-propyl)quinuclidine-1-ium] (poly PTQ) for real-time fluoro-metric determination of the activity of ATPase.[13] The fluorescence of Poly PTQ is quenched upon the addition of ATP, but recovers when the ATP is hydrolyzed by the ATPase. Conversion of ATP to ADP brings about a change in both fluorescence and color (pink-red to yellow).

Knaapila et al. explored the effect of increasing concentration of sodium dodecyl sulfate (SDS) in the solution on the structural reorganization and photophysical properties of poly[3-(6-trimethylammoniumhexyl)thiophene]bromide (P3TMAHT).[119] This work provided vital information on the influence of surfactant complexation on both the solution structure and the photophysical properties of water-soluble PTs. The mole fraction of SDS defined the supramolecular arrangement formed by the interaction of SDS and P3TMAHT. Wang et al. reported the use of a poly[*N,N,N*-trimethyl-4-(thiophen-3-ylmethylene)-cyclohexanamiium chloride] (PTCA-Cl) as a colorimetric and fluorometric probe for the detection of anionic surfactants.[120] This assay could detect concentrations as low as 1 nM. However, it was not specific against detergents.[120] They further reported a colorimetric and fluorometric probe for the detection of I⁻ ions and anionic surfactants with high selectivity and sensitivity using poly[3-(1,1′-dimethyl-4-piperidinemethylene)thiophene-2,5-diyl chloride] (PDPMT-Cl).[121] The two PTs, PTCA-Cl and PDPMT-Cl, differ in the cationic part of the PTs sidechain, which clearly highlights the importance of the functional groups of the PTs in developing selective optical sensors to yield different optical responses for different detergents.

Surfactants. Surfactants are significant contributors to water pollution because of their extensive use in domestic and industrial cleaning processes. Considering the need to detect these detergents, facile and cost-effective optical assays have been developed. The electrostatic, hydrophobic packing, hydrogen bonding, and ion-pairing interaction between PT and detergents play a crucial role in the formation of supramolecular aggregates that generate optical responses.

Evans et al. reported the use of P3TMAHT to develop an optical sensor for selectively identifying anionic surfactants based on their structural subgroups.[122] Their findings suggested that the subtle differences in the surfactant mole fraction and chemical structure (e.g., chain length, headgroup charge density) yielded variations in the range and type of complexes formed, which directly correlated to a unique colorimetric and fluorometric fingerprint response for the detergents (Figure 9).

These studies suggest that a detailed investigation of the specific nature and mechanism of interactions between the side-chains of the PTs and the analyte molecule is crucial. Such knowledge could help in designing PTs for selective and sensitive optical sensors with a tailored spectral response.

## Optical Biosensors for Detection of Nucleotides.
Nucleotides have phosphate groups which give them a net negative charge, and the nucleotide bases (i.e., adenine, thymine, cytosine, guanine and uracil) form hydrogen bonds. Since electrostatic interactions primarily govern the interaction of PTs with analyte molecules, a family of cPTs have been used for developing optical sensors. cPTs have side chains with a positively charged functional group at the end, which interact with the negative phosphate groups of nucleotides and form hydrogen bonds with the nucleotide bases.[29,70] This turns the use of cPTs into a promising approach for developing optical assays for nucleotides. The binding of a ssDNA sequence to cPTs causes the aggregation of the conjugated backbone resulting in a color change and a significant decrease in fluorescence of the cPTs. In the presence of a complementary sequence, the nucleotides interact preferentially with each other instead of the cPTs, resulting in a significant recovery of fluorescence and color. This aggregation and disaggregation of
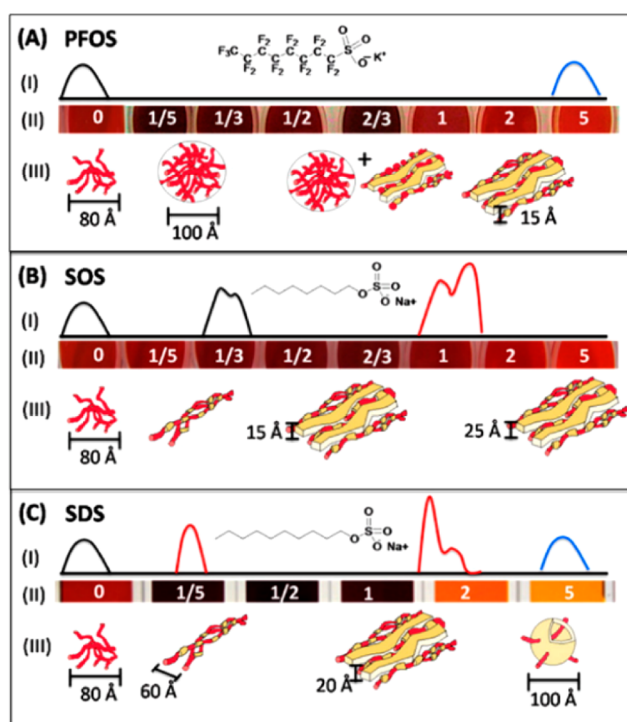
Figure 9. Schematic representation of the optical response and correlation between the structure and supramolecular organization of P3TMAHT−surfactant. The detergents used are (A) potassium heptadecafluoro-1-octanesulfonate, PFOS; (B) sodium octyl sulfate, SOS; and (C) sodium dodecyl sulfate. (I) Fluorometric response is represented by changes in emission band shape or wavelength relative to isolated P3TMAHT (black line). Blue and red shifts in λ_{em} are indicated by the emission band color. (II) Observed colorimetric response as a function of mole fraction. (III) Proposed P3TMAHT−surfactant complex structures in aqueous solution. The red and yellow areas refer to P3TMAHT and surfactant, respectively. Reprinted with permission from ref 122. Copyright 2012 American Chemical Society.

the cPT backbone is the basis of the majority of the optical sensors for the detection of nucleotides.

Ho et al. suggested that the cPTs have distinct electrostatic interactions with ssDNA and dsDNA, resulting in distinct conformational changes (Figure 10).[123] These conformational changes form the basis of the different optical responses of cPTs in the presence of ssDNA and dsDNA. This approach can easily distinguish between a perfect complementary match and oligonucleotides containing 1−2 base pair mismatches. When interacting with ssDNA, the cPTs form a highly
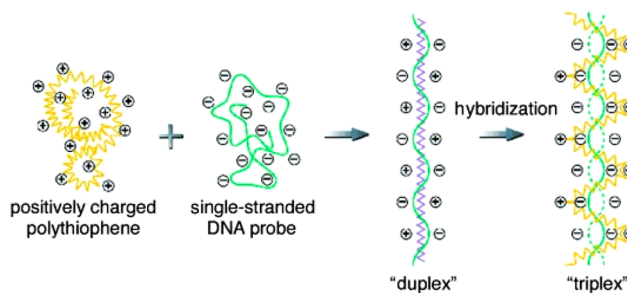

Figure 10. Schematic of the formation of PT/ssDNA duplex and PT/dsDNA triplex forms. Reprinted with permission from ref 123. Copyright 2002 John Wiley and Sons.

conjugated double helix (duplex) with a planar conformation. However, with the addition of complementary sequences, they form a less conjugated triplex structure with planar conformation (Figure 10). This study suggested that this difference between the conformational structure of the cPTs backbone in the duplex and triplex configuration is the basic principle of the detection approach, which yields an optical (colorimetric or fluorometric) response.

Magnieto et al. studied the influence of side-chain functional groups of cPTs and sequence of DNA on the chirality of cPTs.[70] The interaction of ssDNA and cPTs with different functional groups showed ICD in the cPTs absorbance region (400−600 nm). The intensity of ICD peaks depends on the charge and localization of cationic groups of cPTs, with smaller and more localized charge groups showing the highest ICD peak intensities. ssDNA has a higher affinity for cPTs as compared to dsDNA because the bases of ssDNA provide higher hydrophobicity for interaction via π-type interactions. In another study, they also suggested that the interactions between cPTs and DNA depend upon the nature of the DNA sequence, topology, and length (Figure 10).[123]

Xinrui Duan et al. also used the interactions between nucleotides and cPTs to detect genetic modifications such as single nucleotide polymorphisms (SNP), DNA methylation, and other lesions in nucleotide sequences.[28] Unlike the previously reported assays using a single PT, Rajwar et al. used a combination of homo- and co-PTs to develop an assay for miRNA detection.[86] PTs having different functional groups in a single polymer yielded better optical responses for miRNA detection in comparison to their homopolymers. Upon binding to target miRNA, cPTs form a duplex. However, when a peptide nucleic acid (PNA) is present along with target miRNA, they form a triplex with the cPT. These structural differences yielded distinguishably higher fluorescence responses as compared to miRNA-cPTs duplexes. They also created a digital array for miRNA assay to compare different homo- and copolymer of PTs (Figure 11).[86]
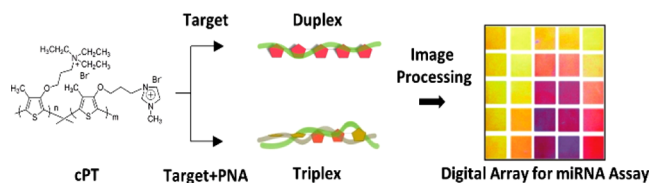
Figure 11. Schematic of cPT/cPT−target miRNA duplex and PT−PNA−target miRNA triplex formation using homo- and copolymers. Representation of the optical response in a digital array format for comparison.[86] Reprinted with permission. Copyright 2016 American Chemical Society.

Most of the cPT-based optical assays detect nucleotides in solutions. These solution-based assays recently have been translated into membrane/paper-based assays for easier use. Rajwar et al. translated the PT-based assays in solution to a membrane-based flow-through colorimetric assay to detect and distinguish nucleotide biomarkers such as lung cancer-associated microRNA (mir21) and hepatitis B virus DNA (HBV-DNA) (Figure 12A−D).[87] The sensing approach was based on the fact that the optical response of cPTs is different upon complexation with target nucleic acids in the presence or absence of their corresponding complementary PNAs. In this study, the solution-based assay was translated to poly-
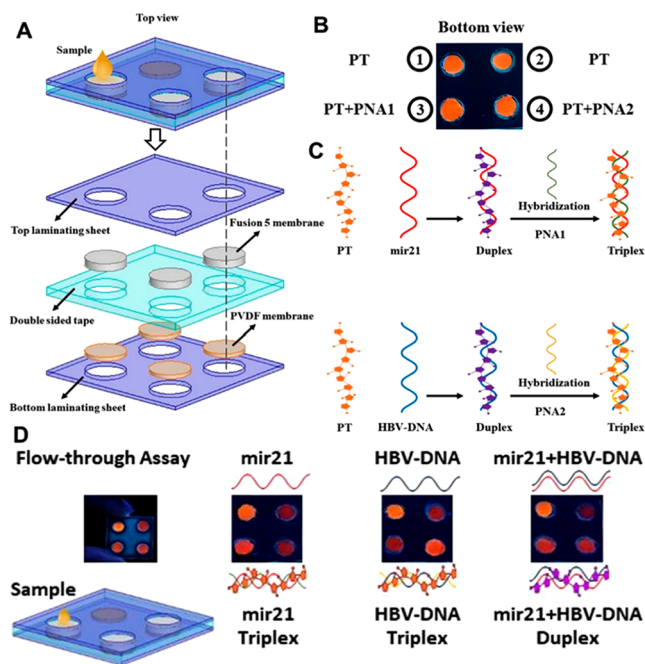
Figure 12. (A) Illustration of the flow-through device. (B) Response of the flow-through device showing a colorimetric response of PT impregnated PVDF membranes (bottom view) and number represents: 1, reference; 2, for monitoring duplex response; 3, for mir21; 4, HBV-DNA assay. (C) Schematic representation of sensing strategy of mir21 and HBV-DNA through the duplex and triplex formation with PT in the absence and presence of their complementary PNAs. (D) Response of the flow through device in the presence of the two biomarkers used for proof-of-concept testing. Reprinted with permission from ref 87. Copyright 2019 Elsevier.

(vinylidene fluoride) (PVDF) membrane-based assay. The flow-through colorimetric assay enabled the detection of mir21 and HBV-DNA in plasma without requiring tedious sample pretreatment and cleanup protocols for potential use in point-of-care diagnosis. The diagnostic potential of PTs has been recently demonstrated by the detection of SNP associated with Familial Mediterranean Fever (FMF) with non-amplification-based nucleic acid assay (Figure 13).[88] This study described a solution based assay of polythiophene polyelectrolyte and target genes.

The flow-through colorimetric assay offers great potential for rapid point-of-care disease diagnosis as well as nucleotide detection in plasma by preventing the need for any sample pretreatment processing. The ability of cPTs to interact with
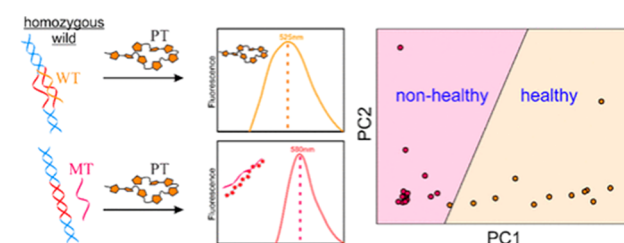

Figure 13. Schematic of a non-amplification-based nucleic acid assay for the detection of SNP associated with FMF using cationic polythiophene. The fluorescent PTs are analyzed using principal component analysis (PCA), providing a clear separation of healthy and patient individuals. Reprinted with permission from ref 88. Copyright 2021 American Chemical Society.

nucleotide biomarkers in the presence and absence of PNA samples can be developed as adhesive patches to monitor nucleotide biomarkers continuously in biological fluids (i.e., sweat, plasma, urine, and others). However, nonspecific electrostatic interactions of PTs with various molecules in the samples result in a higher signal-to-noise ratio. Thus, using specifically designed PTs and flow-through assemblies in the assay would undoubtedly help circumventing these issues.

Optical Biosensors for Detection of Protein and Enzymes. Proteins and peptides have always been essential targets for developing point-of-care optical sensors. Hence, various strategies have been used to develop facile colorimetric sensors for proteins and peptides using PTs.

Ho et al. developed an optical assay for detecting proteins and biomolecules. They used a cPT (poly(3-alkoxy-4-methylthiophene)) and an aptamer (ssDNA) to develop an optical sensor for detecting human α-thrombin.[124] The aptamers bind explicitly to human α-thrombin and undergo a conformational transition from an unfolded to a folded structure. This conformational change in the aptamer can be detected by cPT forming a new complex with a distinct optical (colorimetric or fluorometric) signal without any labeling of the aptamer or the human α-thrombin (Figure 14).[124]
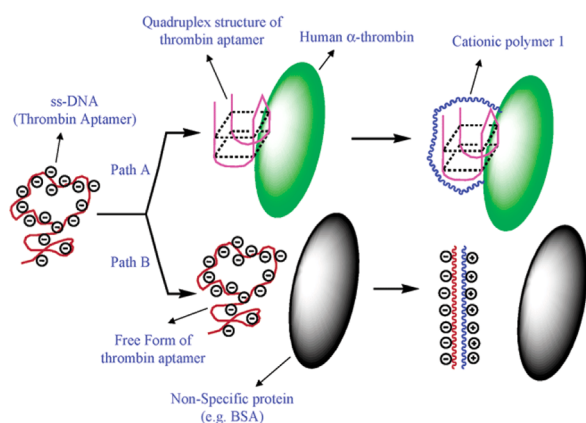
Figure 14. Illustration representing the specific detection of human α-thrombin by use of ss-DNA thrombin aptamer and cPT. Reprinted with permission from ref 124. Copyright 2004 American Chemical Society.

In a similar manner, Abérem et al. used a combination of DNA aptamers and cationic PTs in microarray format on a glass slide along with human thrombin for proof-of-concept demonstration (Figure 15).[19] Mixing cPTs and ssDNA aptamer formed neutral rigid-rod moieties that self-assemble into nanoaggregates resulting in a significant quenching of the fluorescence. Notably, the ssDNA aptamer undergoes a conformational transition from an unfolded to a folded (G-quadruplex) structure upon the addition of human thrombin, causing a significant increase in the fluorescence. On the contrary, a nonspecific protein did not bring about any such change. These approaches used a protein-specific aptamer for the detection of protein molecules. Interestingly, a few studies suggest that the direct detection of proteins is feasible based on the interaction of PT and the target protein molecules.[18,25,48,125,126]

Protamine is a protein molecule with various therapeutic applications, which becomes toxic at higher concentrations.[25,127] The standard approach for protein concentration
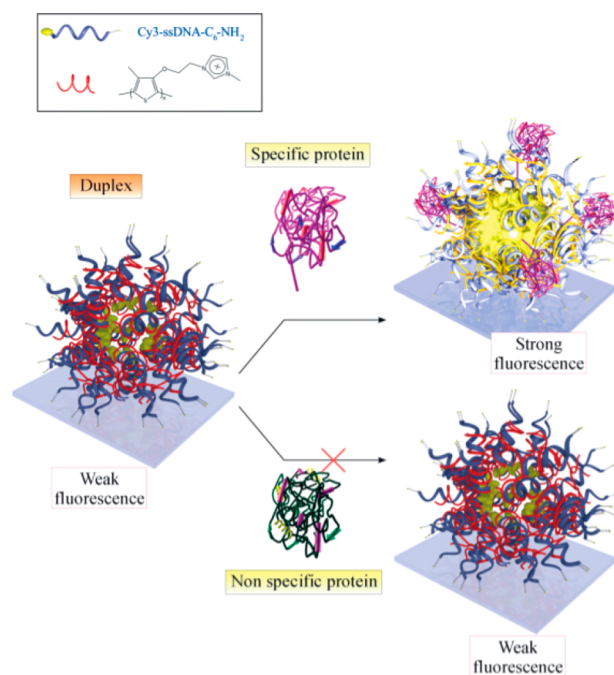
Figure 15. Schematic representation of the specific recognition of target proteins by ssDNA−PT aptamer duplex aggregates performed on glass slides. Reprinted with permission from ref 19. Copyright 2006 John Wiley and Sons.

determination (i.e., UV-absorption at 280 nm) is inefficient in this case because of the lack of aromatic amino acids in their primary structure. Wu et al. reported a novel approach for the colorimetric and fluorescent detection of protamine using an anionic PT, poly(2-(2-(4-methylthiophen-3-yloxy)ethyl) malonate acid (PMTEMA).[25] Protamine is arginine-rich, which turns it into a strongly basic protein. Negatively charged PMTEMA interacts with a highly positively charged protamine, forming a complex through multiple electrostatic interactions. The complex formation produces conformational and aggregational changes in the PT backbone, with a corresponding fluorescence quenching and visual color change of the solution. This visual optical response makes PMTEMA a promising probe for colorimetric and fluorometric detection of protamine (Figure 16).[19] Interestingly, if the protamine molecules are digested into smaller fragments using a protease, trypsin, then these fragments can no longer bring about the optical response. They further used this approach to detect the
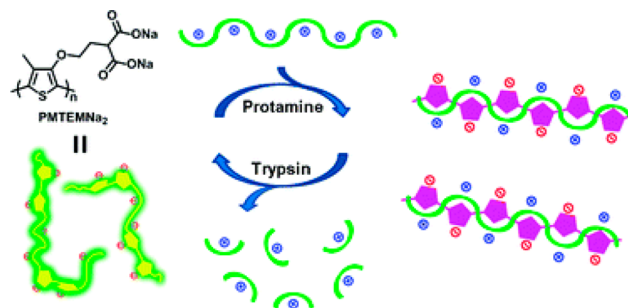
Figure 16. Chemical structure of PMTEMA and schematic illustration of the detection of protamine and trypsin. Reprinted with permission from ref 25. Copyright 2013 Royal Society Of Chemistry.

activity of trypsin. Proteases also have been reported as essential biomarkers for several diseases. Therefore, designing peptide substrates for these proteases that selectively show optical responses with PTs offer huge potential in point-of-care assaying.

Abnormal levels of cysteine (Cys) or homocysteine (Hcy) are related to many complications, such as slowed growth in children, depigmentation of hair, edema, Alzheimer's disease, and cardiovascular diseases.[128−130] Yao et al. reported an optical assay for the selective detection of Cys and Hcy using PMTPA.[125] 2-Cyano-6-methoxybenzothiazole (CBT) reacts with Cys and Hcy leading to the formation of luciferin derivatives. These luciferin derivatives with a sizable aromatic plane and negative charges form a complex with cationic PMTPA through ionic self-assembly resulting in color change with a decrease in fluorescence (Figure 17).
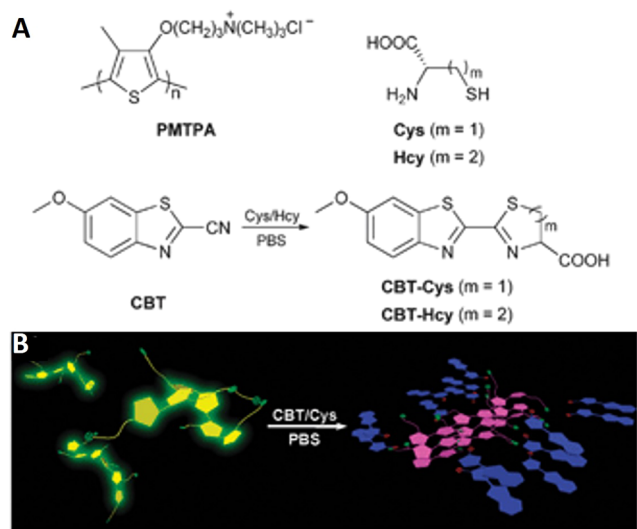
Figure 17. (A) Chemical structure of PMTPA and reaction scheme for the in situ reaction between CBT and Cys (or Hcy) in PBS. (B) ISA process of PMTPA in the presence of CBT and Cys (or Hcy). Reprinted with permission from ref 125. Copyright 2011 Royal Society Of Chemistry.

Nelson et al. demonstrated the use of carboxylic acid-functionalized PT (PT$_{CA}$) for the detection of diamines based on the electrostatic and hydrophobic interactions between them.[48] PT$_{CA}$ produced unique spectral patterns in response to structurally similar diamines (Figure 18). Various factors determined the optical response of PTs: planarization/deplanarization of the PT backbone, $\pi-\pi$ interactions between the PTs chain, and scattering of visible light because of aggregation. These factors depend on PT−diamine interactions, the length and rigidity of the bonds between the amine moieties. Their findings suggested that highly accurate identification can be achieved regardless of the amount of diamine, because the response relies on the composite responses from the assembly across the entire spectrum and not because of a variation in a single wavelength. Thus, instead of just using absorbance and fluorescence to distinguish among various diamines, a multivariate statistics approach to deconvolute subtle differences in the resulting spectra was adopted. The analytes were identified with an accuracy over 99% using this approach. Maynor et al. further improved the approach by identifying and differentiating 22 structurally
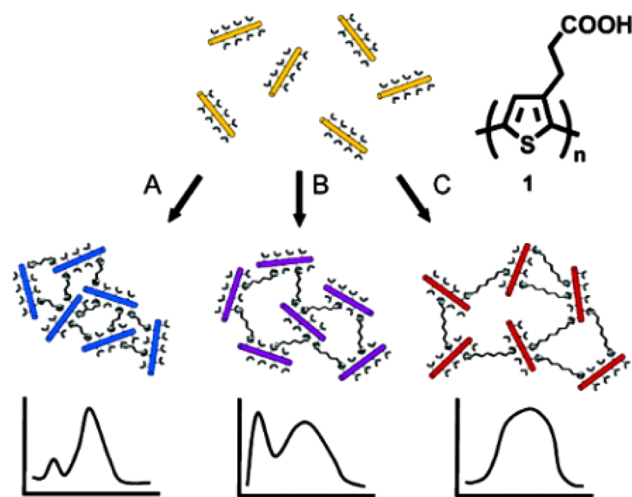
Figure 18. Illustration representing the aggregative interactions between PT 1 (colored rods) and different diamine analytes (A−C), resulting in the formation of various colored aggregates depending on added diamine, which is depicted by the different colored rods and simulated absorbance traces. Reprinted with permission from ref 48. Copyright 2006 American Chemical Society.

similar and biologically relevant amines with 97% accuracy.[126] They used statistical analysis on an array of wavelengths to differentiate multiple samples using a single PT. For example, LDA was used to identify the patterns in the spectral fingerprints obtained from the interactions of carboxy-functionalized PTs and the amine-containing compounds. This approach also was applied to detect biogenic amines in fish.[126] An array of PT was further developed to improve accuracy and differentiate multiple analytes.[18]

Miranda et al. used an array of conducting polymers to detect multiple protein analytes.[18] The conducting polymers used in the study had different charged functional groups which could interact with proteins via multivalent interactions. These structural features provided tremendous binding diversity upon interaction with protein analytes, hence generating distinct fluorescence response patterns for protein discrimination (Figure 19). Patterns in the distinct fluores-
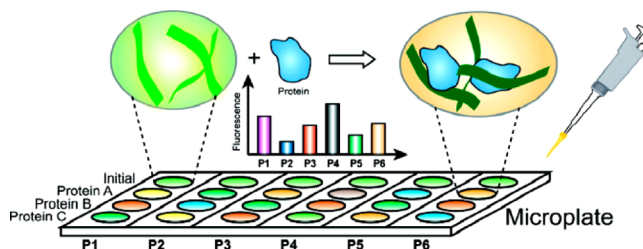
Figure 19. Illustration of array-based sensing of proteins using conjugated polymers. Reprinted with permission from ref 18. Copyright 2007 American Chemical Society.

cence response of PTs for different protein samples were analyzed using LDA.[18] Patterns for 17 proteins were analyzed and 97% identification for unknown samples was achieved.

Taken together, the use of pattern identification approaches and PT arrays for the detection of multiple protein analytes can certainly help to develop point-of-care sensors to detect and distinguish multiple analytes simultaneously.

Optical Biosensors for Detection of Bacterial Pathogens. Bacterial and viral pathogens are a significant cause of water and food contamination every year. Hence, detection of these pathogens with point-of-care assays is important. Numerous approaches based on nucleotide and antibodies have been developed. However, ease-of-use and field deployability are significant limitations for most of them.

Plante et al. reported the use of cPT (poly(3-lkoxy-4-methythiophene), AH-35) for culture-independent, direct, rapid, and yet straightforward detection of microbial particles in water.[31] The interaction of AH-35 PT with the negatively charged bacterial surface results in a significant decrease in fluorescence (Figure 20). They used *E. coli* for a proof-of-
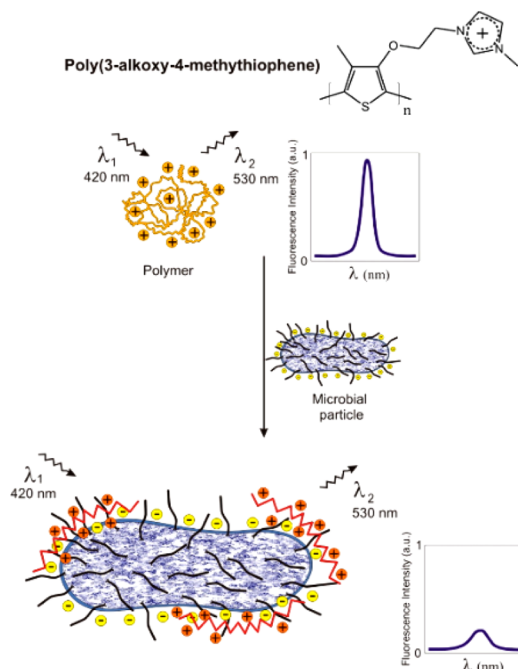
Figure 20. Schematic description of the method used for the detection of microbial particle. When the cPT is alone, its fluorescence is optimal. After adding a microbial particle, a diminution of fluorescence without any wavelength shift is observed. Reprinted with permission from ref 31. Copyright 2013 American Chemical Society.

concept demonstration. The outer membrane composition of bacterial pathogens differs in composition. Thus, designing the PTs with specific side chains that interact differently with the membrane or using an array of PTs can help to develop an approach to detect and distinguish multiple bacterial pathogens simultaneously.

Li et al. reported the development of a rapid, simple, and high-throughput screening methodology using antimicrobial photosensitizers (PSs).[32] This approach was based on the quenching of the fluorescence of poly[3-(3′-*N,N,N*-triethylamino-1′-propyloxy)-4-methyl-2,5-thiophene hydrochloride] (PMNT) upon interaction with the bacterial pathogens. The PSs which are not effective in sensitizing would result in exponential bacteria growth. Hence, the coating of the bacterial surface tightly by PMNT through electrostatic and hydrophobic interactions resulted in aggregation and fluorescence quenching of PMNT, whereas effective PSs lead to original and strong fluorescence of PMNT (Figure 21).
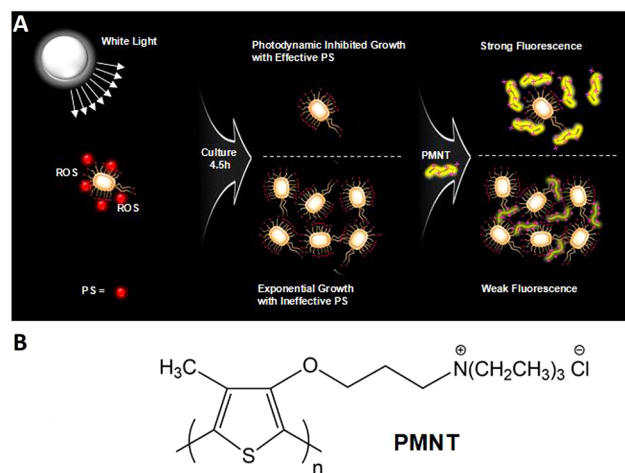
Figure 21. (A) Schematic representation of a PMNT-based platform for screening of photodynamic antimicrobial chemotherapy properties of PSs and (B) chemical structure of PMNT. Reprinted with permission from ref 32. Copyright 2015 American Chemical Society.

The use of PTs for the detection of bacterial pathogens based on their surface properties was further developed by Bai et al.[33] They reported on the use of a PT-based system for differentiating virus particles and other microbes.[33] Moreover, they employed a combination of a cPT and barrel-shaped macrocyclic molecular cucurbit[7]uril (CB[7]) as reporter molecules. The two reporters (PT and PT/CB[7]) used in the assay interact differently with virus and bacterial pathogens, producing a distinct optical (fluorescence) response. Standard LDA was used to find patterns to distinguish between viruses and bacterial pathogens (Figure 22). This work is yet another
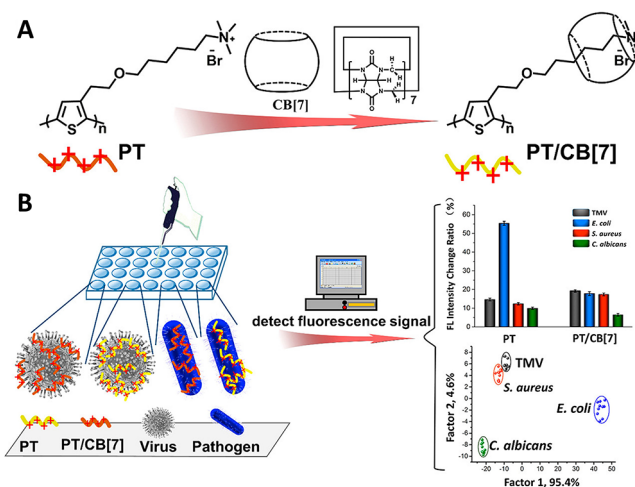
Figure 22. (A) Schematic representation of the assembly process of the supramolecular sensor system. (B) Schematic illustration of the approach used for detection and discrimination between the virus and bacterial pathogens. Reprinted with permission from ref 33. Copyright 2018 American Chemical Society.

example that designing specific and selective PTs-based reporter molecules or biological probes could promote the application of conjugated polymer materials in biosensor development.

Han et al. recently reported on the use of conducting polymers and peptide complexes to detect and distinguish bacterial pathogens in the urine.[30] They suggested using yet

another negatively charged conducting polymer reporter material, poly(*para*-phenylene ethynylene) (PPE), which forms electrostatic complexes with four positively charged antimicrobial peptides (AMP). The complex between PPE and AMPs partially quenched the fluorescence of the PPE. However, AMP-PPE complexes interact distinctly different with the surface of bacterial pathogens (Figure 23). LDA was
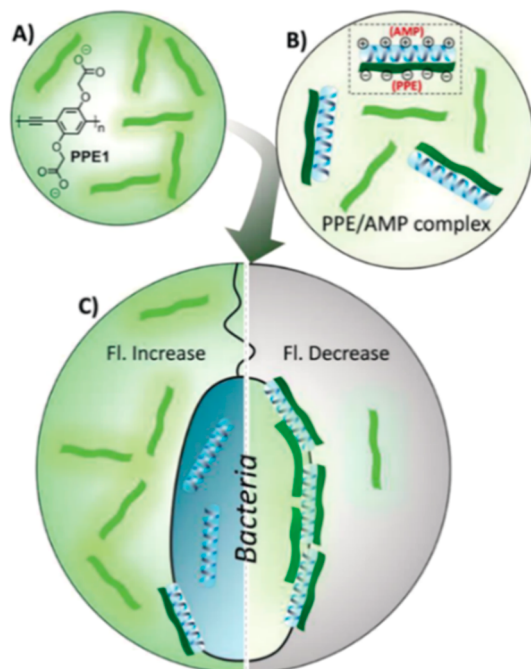
Figure 23. Schematic illustration of PPE/AMP complex-based assay for the discrimination of different bacteria. (A) Chemical structure of conducting polymer PPE1. (B) Representation of electrostatic complex formed between negatively charged PPE and positively charged AMP resulting in quenching of the PPE fluorescence. (C) Addition of bacteria to the PPE/AMP complex leads to the fluorescence increase caused by the displacement of the indicator molecule (left) or results in the fluorescence decrease caused by the aggregation of the PPE/AMP complex on the surface of bacteria (right). Reprinted with permission from ref 30. Copyright 2017 John Wiley and Sons.

used to evaluate the fluorescence response of different bacterial pathogens. The LDA analysis helped to group the bacterial species and strains into clusters according to staining properties (Gram-positive and Gram-negative) or genetic similarity (genus, species, and strain).

Sinsinbar et al. recently reported an optical assay for the detection of bacterial pathogens based on the activity of the bacterial membrane proteases and interaction of PTs with protease substrate.[131] They used an optimized peptide substrate for an outer membrane protease, OmpT, present on *E. coli,* and polythiophene acetic acid (PTAA) as the reporter PT.[131] The protease substrate, when interacting with PTAA, results in a blue shift in the absorbance spectrum and an increase in fluorescence (Figure 24). However, in the presence of *E. coli,* the peptide substrate was cleaved by OmpT, and the cleaved peptide fragments were not capable of bringing about any change in the absorbance and fluorescence spectra. This assay is a functional and highly specific assay using anionic (COO⁻ modified) PT, unlike the previously reported electrostatic or hydrophobic interaction-based assays.[30,33]
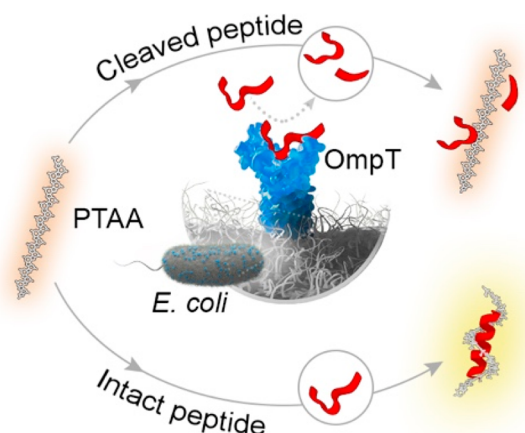
Figure 24. Schematic illustration of OmpT-based *E. coli* sensing with anionic polythiophene and unlabeled peptide substrate. Reprinted with permission from ref 131. Copyright 2020 John Wiley and Sons.

Various PTs have been used as reporter molecules in different assay formats, and a few have been discussed above. Alternative bioanalytical strategies also have been reported using PT and related conjugated materials, for instance, in imaging and diagnosis, which will be discussed in the next section.

RECENT DEVELOPMENTS AND PERSPECTIVES

Despite the extensive use of PTs as reporter molecules in developing optical biosensors, there are a few limitations that require future attention. One major limitation is related to the synthesis of PTs. Synthetic PTs are mostly polydisperse, which affects the conjugation length of the PTs. This results in broad and diffuse responses, as both the emission and absorption spectra critically depend on the length of the thiophene backbones. The polydispersity of PTs not only affects the optical responses of PTs, but also their abilities to interact with analytes.

There is always a batch-to-batch variation in synthesis, and even the characterization of the PTs is difficult. For molecular weight estimation, gel permeation chromatography is commonly used. However, the polystyrene standards used for calibration possess very different geometrical restraints. Hence, the calculated and actual sizes can be quite different. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) previously has been used for characterization of the size distribution, however, with its own limitations. First, not all PTs can be ionized effectively. Second, the polydisperse chains of PTs would have different degrees of ionization, affecting the accurate estimation of chain length distribution. These limitations make it challenging to obtain PTs of consistent sizes. Several groups have utilized luminescent conjugated oligothiophenes (LCOs) of fixed chain size to prevent these issues. These LCOs are synthesized by adding required units one-by-one. Although during the synthesis of LCOs, few purification steps are required, unlike PT synthesis, LCOs have reported several advantages compared to PTs,[43,132,133] because LCOs with a monodisperse size have consistent absorption and emission spectra. PT optical response is mainly dependent on the electrostatic interactions between the analyte and PT. However, false positives can occur because of nonspecific interactions between charged biomolecules and PTs. There are fewer electrostatic/

hydrophobic interactions between LCOs and target molecules compared to PTs because of the fixed chain size and charges. Therefore, LCOs show higher specificity to molecules and less pronounced nonspecific interactions with charged molecules as compared PTs.[133] LCOs also have a higher quantum yield as compared to PTs because of less aggregation-induced self-quenching. Although these properties make LCOs a better optical reporter as compared to PTs, they have not been explored extensively as a reporter to develop optical biosensors for biomolecules like nucleotides, proteins, peptides, etc.[134,135] However, Nilsson et al. have extensively used LCOs to study protein aggregation diseases, cancer, and bacterial infection.[95,96,136−139] They also used LCOs to distinguish the amyloid-like fibrillar state and the native protein.[132,133,140,141] At the same time, well-defined conjugated oligoelectrolytes are also being used for developing optical sensors for detection of bacterial pathogens, DNA, proteins, and other molecules.[142−145] Use of LCOs as selective and specific reporter molecules in developing optical sensors for point-of-care assays is a potential way forward to improve upon the limitations of PTs.

The basic molecular mechanisms behind the induced optical responses of PTs are not very well understood. It is universally agreed that mostly electrostatic and hydrophobic/hydrophilic interactions are established between PTs and analytes. However, the effects of these interactions are not clearly understood in relation to the structural and optical properties of π-conjugated PTs. Previous reports suggest selective detection of small ions and molecules by using PTs with side-chains having different functional groups. However, the reasons for PTs to interact with similarly charged species still remain unanswered. Improvement in understanding these interactions would definitely enable designing PTs with side-chains and functional groups specific to an analyte. Molecular dynamics simulations have been performed to study the interactions between PTs and analyte, but these studies are still not well developed for different PTs and target analytes.[29] The effects of side-chain length, saturation and unsaturation of side-chain, functional groups, and various other parameters need to be characterized and understood in detail in order to design and synthesize PTs that specially bind to the analytes. Rittmeyer et al. studied the substitutions at the side chain of PTs and the corresponding effect on the electronic structure of thiophenes using periodic density functional theory calculations.[146] The effects of the substitutions were far more complex and could not be explained just based on simple electron removal or addition. Although these molecular modeling and simulation studies are still in their infancy, the development of these studies can be of great importance to design novel PTs/LCOs advance the understanding of their interactions with analyte molecules.

Macromolecules like proteins or bacterial pathogens have multiple distinct interaction points with PTs. Hence, designing selective and specific PTs for these larger molecules is very challenging. However, by using a combination of arrays of PTs along with LDA analysis, it could be possible to develop strategies for specific detection of macromolecules. The assays could be linked to cellphone-based data capture and analysis for point-of-care applications. Another critical aspect of the PT-based assays is that they are being developed as facile optical assays for point-of-care diagnosis. However, most of the reported PT-based sensing reports are on solution-based assaying. Thus, the translation of these solution-based assays into paper, lateral-flow, or dipstick formats would be essential for the development of future point-of-care solutions. Our group has worked on the translation of solution to membrane- and paper-based assays for point-of-care applications.[87,147,148] In addition, translation into paper-based assays also reduce the need for tedious sample pretreatment steps as filtration and sample enrichment strategies can be easily integrated with such platforms.

Fluorescent soft conjugated polymer nanoparticles and dots have changed routines in bioimaging, diagnostics,[149,150] and drug therapy studies.[150] The soft conjugated polymer nanoparticles exhibit reversible chain deformations and displacement by the conformation transition of their polymer backbone, causing significant changes in photophysical properties.[151,152] Moreover, they provide high emission intensity, chemical modularity, and biocompatibility.[150] By their property, soft nanoparticles also exhibit penetration capability in both inter- and intracellular milieu. Recent reports discuss the use of conjugated polymer nanoparticles (CPNs) and polymer dots (Pdots) in in vitro and in vivo imaging and diagnosis.[153] The single-chain Pdots in reduced diameter (<10 nm) have shown improved quantum yield, photostability, and colloidal stability[153−155] as compared to CPNs. Therefore, in biological applications, single-chain Pdots are expected to show unambiguous advantages.[156−158] Recently, Ozenler et al. demonstrated the use of single-chain Pdots for differentiation of cancer and healthy cells in coculture medium.[159] Hepatocellular carcinoma (HuH-7 cells) and healthy adult liver epithelial cells (THLE-2 cells) were used to assess the accumulation of Pdots in the cells (Figure 25A). Figure 25B (upper panel) shows HuH-7 cells where Pdots were localized in the nuclei, whereas they accumulated in the cytoplasm and around the nuclei of THLE-2 cells (Figure 25B, lower panel). The selective translocation of Pdots into the nuclei of cancer cells was confirmed by a coculture experiment for HuH-7 and THLE-2 cells (Figure 25D−G).

A recent review on Pdots summarizes major developments in bioimaging and sensing. In particular, narrow emission and NIR region- and agregation-induced emission of semiconducting polymer nanoparticles were carefully reviewed.[160] In yet another study, Chiu et al. has shown that photoswitchable Pdots can be used to paint and sort cells selectively that enables genetic analysis of selected cells (Figure 26).[161]

In vivo dynamic monitoring by implantable Pdots has been used to exemplify this detection strategy by using glucose-oxidase-functionalized polymer dots. The Pdot−enzyme assembly after subcutaneous implantation provided an efficient signal that was transdermally detectable and continuously responsive to blood glucose fluctuations for up to 30 days. Furthermore, their results offered high selectivity, large dynamic range, and reversible glucose detection in cell and tissue environments using Pdots.[162] Pdots also were utilized for photoacoustic imaging. For instance, Podts emitting in the second near-infrared (NIR II) region were successfully applied for photoacoustic imaging of orthotopic brain tumors. For example, Pdots emitting at NIR II offered efficient skull penetration and improved signal/background ratio using a 1064 nm laser.[163] These attractive advantages of Pdots provide effortless translation for precise determination of surgical borders in cancerous tissues and for discriminating cell histological examinations.

We see huge potential for nanoscale PT-based reporters, with improved quantum yield, biocompatibility, and colloidal
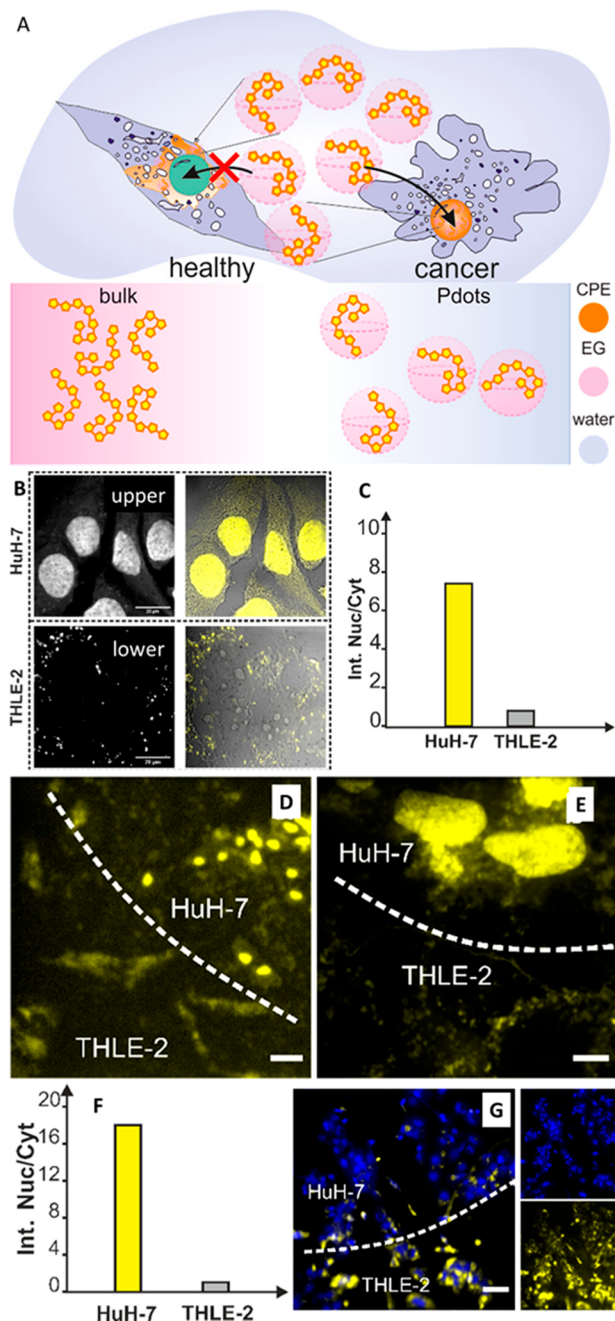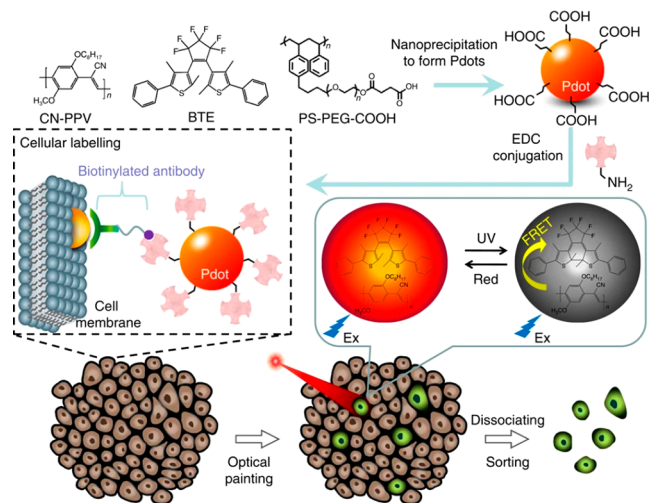
Figure 25. (A) Schematic illustration of accumulation of Pdots in the cells and nanophase separation between ethylene glycol (EG) and water during Pdot formation. (B) Bright field and fluorescent images of (upper) HuH-7 and (lower) THLE-2 cells. (C) Fluorescence intensity ratio of nucleus to cytoplasm. Images of cocultured THLE-2 and HuH-7 cells at (D) 20× magnification (scale bar: 50 μm) and (E) 100× magnification (scale bar: 10 μm). Images captured after DAPI (F) and Pdot (G) staining (inset figures showing DAPI only (upper) and Pdot only (lower)). Reprinted with permission from ref 159. Copyright 2019 American Chemical Society.

Figure 26. Schematic illustration of the process of Pdot formation, cellular labeling using Pdots, and "painting" of labeled cells with light, followed by the sorting and isolation of painted cells. Reprinted with permission from ref 161. Copyright 2016 Springer Nature.

continuously changing needs and challenging problems in detection, imaging, and theranostic applications require PTs and related polymer particles with improved properties. The past and recent studies have shown that in vitro ultrasensitive detection of target analytes is feasible by using PTs. However, the synthesis and application of nontoxic and biocompatible PT probes that are injectable/digestible are still challenging. In clinical practice, it is an urgent need to explore noninvasive as well as biocompatible yet functional PT assays for enabling real-time detection in the living systems, including, for example, continuous monitoring of biomarkers for viral and bacteriological infections. Moreover, the automated genomic and proteomic instruments are an integral part of the state-of-the-art diagnostic processes, and we envision huge potential of PTs as optical probes in these array type assays in conjunction with LDAs. Bioconjugation of PTs with recognition elements that are specific for biomarkers appears as another potential future research direction since the rapid detection without pretreatments will be of prime interest for their application in resource-limited settings. PTs have already generated new paradigms such as naked eye detection and near-zero-cost diagnostic tools. Furthermore, PTs exhibit substantial potential in continuously monitoring, wearable, and implantable/digestible devices for near-future diagnostic applications.

■ AUTHOR INFORMATION

Corresponding Author

Bo Liedberg − *Centre for Biomimetic Sensor Science, School of Materials Science Engineering, Nanyang Technological University, Singapore 637553;* orcid.org/0000-0003-2883-6953; Email: bliedberg@ntu.edu.sg

Authors

Gaurav Sinsinbar − *Centre for Biomimetic Sensor Science, School of Materials Science Engineering, Nanyang Technological University, Singapore 637553;* orcid.org/0000-0001-7720-7198

Alagappan Palaniappan − *Centre for Biomimetic Sensor Science, School of Materials Science Engineering, Nanyang Technological University, Singapore 637553*

stability, to replace/complement the aforementioned CPNs and Pdots in bioimaging and related theranostic applications.

In summary, PTs constitute a special class of sensory materials that provide optical signals in response to conformational alterations in their backbone induced by the target analyte. This intriguing property of PTs is heavily exploited in numerous fields of science and technology. However, the

Umit Hakan Yildiz − *Department of Chemistry, Department of Photonic Science and Engineering, and Department of Polymer Science and Engineering, Izmir Institute of Technology, İzmir 35430, Turkey;* orcid.org/0000-0002-6922-4454

Complete contact information is available at:
https://pubs.acs.org/10.1021/acssensors.1c02476

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript

Notes

The authors declare no competing financial interest.

■ VOCABULARY

CPE,Conjugated polyelectrolytes are macromolecules, which have both delocalized π system and charged or ionizable functional groups in their backbone.; Responsive polymers,Polymers displaying explicit changes to chemical, physical or environmental stimuli such as pH, light, temperature, electricity, etc.; Complexation,Polyelectrolyte complexation is the association of negatively and positively charged macromolecules due to electrostatic interactions.; Conformation,The spatial arrangement of the polymer chain that depends on the rotation around single bonds.; Conjugation length,The effective number of monomer units that pursue a continuous overlap of p orbitals.

■ ABBREVIATIONS

CPEs, conjugated polyelectrolytes; PTs, polythiophenes; cPTs, cationic polythiophenes; LCOs, luminescent conjugated oligothiophenes; VOCs, volatile organic compounds; ICD, induced circular dichroism; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; miRNA, microRNA; SNP, single nucleotide polymorphisms; PNA, peptide nucleic acid; HBV-DNA, Hepatitis B Virus DNA; dNTP, deoxynucleoside triphosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; LDA, linear discriminant analysis; SDS, sodium dodecylsulfate; PS, photosensitizers; AMP, antimicrobial peptides

■ REFERENCES

(1) Jiang, H.; Taranekar, P.; Reynolds, J. R.; Schanze, K. S. Conjugated Polyelectrolytes: Synthesis, Photophysics, and Applications. *Angew. Chemie Int. Ed.* **2009**, *48* (24), 4300−4316.

(2) McCullough, R. D. The Chemistry of Conducting Polythiophenes. *Adv. Mater.* **1998**, *10* (2), 93−116.

(3) Inganäs, O.; Liedberg, B.; Chang-Ru, W.; Wynberg, H. A New Route to Polythiophene and Copolymers of Thiophene and Pyrrole. *Synth. Met.* **1985**, *11* (4−5), 239−249.

(4) Andersson, M. R.; Selse, D.; Berggren, M.; Järvinen, H.; Hjertberg, T.; Inganäs, O.; Wennerström, O.; Österholm, J. E. Regioselective Polymerization of 3-(4-Octylphenyl)Thiophene with FeCl3. *Macromolecules* **1994**, *27* (22), 6503−6506.

(5) Tsuchiya, K.; Ogino, K. Catalytic Oxidative Polymerization of Thiophene Derivatives. *Polym. J.* **2013**, *45* (3), 281−286.

(6) Gobalasingham, N. S.; Noh, S.; Thompson, B. C. Palladium-Catalyzed Oxidative Direct Arylation Polymerization (Oxi-DArP) of an Ester-Functionalized Thiophene. *Polym. Chem.* **2016**, *7* (8), 1623−1631.

(7) Hayakawa, T.; Fukukawa, K.-I.; Morishima, M.; Takeuchi, K.; Asai, M.; Ando, S.; Ueda, M. Formation of Regioregular Head-to-Tail Poly[3-(4-Butylphenyl)Thiophene] by an Oxidative Coupling Polymerization with Vanadium Acetylacetonate. *J. Polym. Sci. Part A Polym. Chem.* **2001**, *39* (13), 2287−2295.

(8) Jeffries-El, M.; Sauvé, G.; McCullough, R. D. Facile Synthesis of End-Functionalized Regioregular Poly(3-Alkylthiophene)s via Modified Grignard Metathesis Reaction. *Macromolecules* **2005**, *38* (25), 10346−10352.

(9) Osaka, I.; Mccullough, R. D. Advances in Molecular Design and Synthesis of Regioregular Polythiophenes. *Acc. Chem. Res.* **2008**, *41* (9), 1202−1214.

(10) Chen, T. A.; Wu, X.; Rieke, R. D. Regiocontrolled Synthesis of Poly(3-Alkylthiophenes) Mediated by Rieke Zinc: Their Characterization and Solid-State Properties. *J. Am. Chem. Soc.* **1995**, *117* (1), 233−244.

(11) Li, C.; Numata, M.; Takeuchi, M.; Shinkai, S. A Sensitive Colorimetric and Fluorescent Probe Based on a Polythiophene Derivative for the Detection of ATP. *Angew. Chemie - Int. Ed.* **2005**, *44* (39), 6371−6374.

(12) Llaudet, E.; Hatz, S.; Droniou, M.; Dale, N. Microelectrode Biosensor for Real-Time Measurement of ATP in Biological Tissue. *Anal. Chem.* **2005**, *77* (10), 3267−3273.

(13) Yildiz, U. H.; Sheng, C. W.; Mailepessov, D.; Xueqi, D. C.; Shochat, S. G.; Liedberg, B. Real-Time Determination of the Activity of ATPase by Use of a Water-Soluble Polythiophene. *Anal. Bioanal. Chem.* **2012**, *404* (8), 2369−2375.

(14) Cheng, D.; Li, Y.; Wang, J.; Sun, Y.; Jin, L.; Li, C.; Lu, Y. Fluorescence and Colorimetric Detection of ATP Based on a Strategy of Self-Promoting Aggregation of a Water-Soluble Polythiophene Derivative. *Chem. Commun.* **2015**, *51* (40), 8544−8546.

(15) Li, C.; Shi, G. Polythiophene-Based Optical Sensors for Small Molecules. *ACS Appl. Mater. Interfaces* **2013**, *5* (11), 4503−4510.

(16) Nilsson, K. P. R.; Herland, A.; Hammarström, P.; Inganäs, O. Conjugated Polyelectrolytes: Conformation-Sensitive Optical Probes for Detection of Amyloid Fibril Formation. *Biochemistry* **2005**, *44* (10), 3718−3724.

(17) Herland, A.; Nilsson, K. P. R.; Olsson, J. D. M.; Hammarström, P.; Konradsson, P.; Inganäs, O. Synthesis of a Regioregular Zwitterionic Conjugated Oligoelectrolyte, Usable as an Optical Probe for Detection of Amyloid Fibril Formation at Acidic PH. *J. Am. Chem. Soc.* **2005**, *127* (7), 2317−2323.

(18) Miranda, O. R.; You, C. C.; Phillips, R.; Kim, I. B.; Ghosh, P. S.; Bunz, U. H. F.; Rotello, V. M. Array-Based Sensing of Proteins Using Conjugated Polymers. *J. Am. Chem. Soc.* **2007**, *129* (32), 9856−9857.

(19) Bera Aberem, M.; Najari, A.; Ho, H.-A.; Gravel, J.-F.; Nobert, P.; Boudreau, D.; Leclerc, M. Protein Detecting Arrays Based on Cationic Polythiophene-DNA-Aptamer Complexes. *Adv. Mater.* **2006**, *18* (20), 2703−2707.

(20) Li, K.; Liu, B. Water-Soluble Conjugated Polymers as the Platform for Protein Sensors. *Polym. Chem.* **2010**, *1* (3), 252.

(21) Ho, H.; Najari, A.; Leclerc, M. Optical Detection of DNA and Proteins with Cationic Polythiophenes. *Acc. Chem. Res.* **2008**, *41* (2), 168−178.

(22) Herland, A.; Inganäs, O. Conjugated Polymers as Optical Probes for Protein Interactions and Protein Conformations. *Macromol. Rapid Commun.* **2007**, *28*, 1703−1713.

(23) Nilsson, K. P. R.; Rydberg, J.; Baltzer, L.; Inganas, O. Self-Assembly of Synthetic Peptides Control Conformation and Optical Properties of a Zwitterionic Polythiophene Derivative. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100* (18), 10170−10174.

(24) Nilsson, K. P. R.; Rydberg, J.; Baltzer, L.; Inganäs, O. Twisting Macromolecular Chains: Self-Assembly of a Chiral Supermolecule from Nonchiral Polythiophene Polyanions and Random-Coil Synthetic Peptides. *Proc. Natl. Acad. Sci. U. S. A* **2004**, *101*, 11197−11202.

(25) Yao, Z. Z.; Ma, W. W.; Yang, Y.; Chen, X. X.; Zhang, L. L.; Lin, C.; Wu, H.-C. H.-C.; Hu, X.; Ma, W. W.; Chen, X. X.; et al. Colorimetric and Fluorescent Detection of Protamines with an Anionic Polythiophene Derivative. *Analyst* **2013**, *138* (19), 5572−5575.

(26) Nilsson, K. P. R.; Inganäs, O. Chip and Solution Detection of DNA Hybridization Using a Luminescent Zwitterionic Polythiophene Derivative. *Nat. Mater.* **2003**, *2* (6), 419−424.

(27) Yao, Z.; Feng, X.; Hong, W.; Li, C.; Shi, G. A Simple Approach for the Discrimination of Nucleotides Based on a Water-Soluble Polythiophene Derivative. *Chem. Commun.* **2009**, *405*, 4696−4698.

(28) Duan, X.; Liu, L.; Feng, F.; Wang, S. Cationic Conjugated Polymers for Optical Detection of DNA Methylation, Lesions, and Single Nucleotide Polymorphisms. *Acc. Chem. Res.* **2010**, *43* (2), 260−270.

(29) Rubio-Magnieto, J.; Azene, E. G.; Knoops, J.; Knippenberg, S.; Delcourt, C.; Thomas, A.; Richeter, S.; Mehdi, A.; Dubois, P.; Lazzaroni, R.; et al. Self-Assembly and Hybridization Mechanisms of DNA with Cationic Polythiophene. *Soft Matter* **2015**, *11* (32), 6460−6471.

(30) Han, J.; Cheng, H.; Wang, B.; Braun, M. S.; Fan, X.; Bender, M.; Huang, W.; Domhan, C.; Mier, W.; Lindner, T.; et al. A Polymer/Peptide Complex-Based Sensor Array That Discriminates Bacteria in Urine. *Angew. Chemie - Int. Ed.* **2017**, *56* (48), 15246−15251.

(31) Plante, M. P.; Bérubé, E.; Bissonnette, L.; Bergeron, M. G.; Leclerc, M. Polythiophene Biosensor for Rapid Detection of Microbial Particles in Water. *ACS Appl. Mater. Interfaces* **2013**, *5* (11), 4544−4548.

(32) Li, R.; Niu, R.; Qi, J.; Yuan, H.; Fan, Y.; An, H.; Yan, W.; Li, H.; Zhan, Y.; Xing, C. Conjugated Polythiophene for Rapid, Simple, and High-Throughput Screening of Antimicrobial Photosensitizers. *ACS Appl. Mater. Interfaces* **2015**, *7* (27), 14569−14572.

(33) Bai, H.; Lu, H.; Fu, X.; Zhang, E.; Lv, F.; Liu, L.; Wang, S. Supramolecular Strategy Based on Conjugated Polymers for Discrimination of Virus and Pathogens. *Biomacromolecules* **2018**, *19* (6), 2117−2122.

(34) Li, C.; Numata, M.; Bae, A. H.; Sakurai, K.; Shinkai, S. Self-Assembly of Supramolecular Chiral Insulated Molecular Wire. *J. Am. Chem. Soc.* **2005**, *127* (13), 4548−4549.

(35) Li, B.; Sauvé, G.; Iovu, M. C.; Jeffries-El, M.; Zhang, R.; Cooper, J.; Santhanam, S.; Schultz, L.; Revelli, J. C.; Kusne, A. G.; et al. Volatile Organic Compound Detection Using Nanostructured Copolymers. *Nano Lett.* **2006**, *6* (8), 1598−1602.

(36) Li, H.; Dailey, J.; Kale, T.; Besar, K.; Koehler, K.; Katz, H. E. Sensitive and Selective NO2 Sensing Based on Alkyl- and Alkylthio-Thiophene Polymer Conductance and Conductance Ratio Changes from Differential Chemical Doping. *ACS Appl. Mater. Interfaces* **2017**, *9* (24), 20501−20507.

(37) Wang, F.; Gu, H.; Swager, T. M. Carbon Nanotube/Polythiophene Chemiresistive Sensors for Chemical Warfare Agents. *J. Am. Chem. Soc.* **2008**, *130* (16), 5392−5393.

(38) Tu, M. C.; Svm, H. K.; Thilini, A.; Wallace, L. T. L.; Moochhala, S.; Yildiz, U. H.; Palaniappan, A.; Liedberg, B. Tuning Pendant Groups of Polythiophene on Carbon Nanotubes for Vapour Classification. *Sensors Actuators, B Chem.* **2017**, *247*, 916−922.

(39) Garnier, F.; Tourillon, G.; Barraud, J. Y.; Dexpert, H. First Evidence of Crystalline Structure in Conducting Polythiophene. *J. Mater. Sci.* **1985**, *20* (8), 2687−2694.

(40) Yamamoto, T. Molecular Assembly and Properties of Polythiophenes. *NPG Asia Mater.* **2010**, *2* (2), 54−60.

(41) Nilsson, K. P. R. R.; Andersson, M. R.; Inganäs, O. Conformational Transitions of a Free Amino-Acid-Functionalized Polythiophene Induced by Different Buffer Systems. *J. Phys.: Condens. Matter* **2002**, *14* (42), 10011−10020.

(42) Cui, C. X.; Kertesz, M. Two Helical Conformations of Polythiophene, Polypyrrole, and Their Derivatives. *Phys. Rev. B* **1989**, *40* (14), 9661−9670.

(43) Magnusson, K. *Poly- and Oligothiophenes - Optical Probes for Multimodal Fluorescent Assessment of Biological Processes*; Linköping University, 2015.

(44) Raithel, D.; Simine, L.; Pickel, S.; Schötz, K.; Panzer, F.; Baderschneider, S.; Schiefer, D.; Lohwasser, R.; Köhler, J.; Thelakkat, M.; et al. Direct Observation of Backbone Planarization via Side-Chain Alignment in Single Bulky-Substituted Polythiophenes. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (11), 2699−2704.

(45) Fan, Q. L.; Zhou, Y.; Lu, X. M.; Hou, X. Y.; Huang, W. Water-Soluble Cationic Poly(p-Phenyleneethynylene)s (PPEs): Effects of Acidity and Ionic Strength on Optical Behavior. *Macromolecules* **2005**, *38* (7), 2927−2936.

(46) Zhou, Q.; Swager, T. M. Methodology for Enhancing the Sensitivity of Fluorescent Chemosensors: Energy Migration in Conjugated Polymers. *J. Am. Chem. Soc.* **1995**, *117* (26), 7017−7018.

(47) Langeveld-Voss, B. M. W.; Janssen, R. A. J.; Meijer, E. W. On the Origin of Optical Activity in Polythiophenes. *J. Mol. Struct.* **2000**, *521* (1−3), 285−301.

(48) Nelson, T. L.; O'Sullivan, C.; Greene, N. T.; Maynor, M. S.; Lavigne, J. J. Cross-Reactive Conjugated Polymers: Analyte-Specific Aggregative Response for Structurally Similar Diamines. *J. Am. Chem. Soc.* **2006**, *128* (17), 5640−5641.

(49) Cagnoli, R.; Caselli, M.; Libertini, E.; Mucci, A.; Parenti, F.; Ponterini, G.; Schenetti, L. Aggregation Behaviour of a Water-Soluble Ammonium-Functionalized Polythiophene: Luminescence Enhancement Induced by Bile-Acid Anions. *Polymer (Guildf)* **2012**, *53* (2), 403−410.

(50) Chen, X.; Guan, H.; He, Z.; Zhou, X.; Hu, J. A Sensitive and Selective Label-Free DNAzyme-Based Sensor for Lead Ions by Using a Conjugated Polymer. *Anal. Methods* **2012**, *4* (6), 1619−1622.

(51) Srivastava, S.; Tirrell, M. V. Polyelectrolyte Complexation. *Advances in Chemical Physics* **2016**, *161*, 499−544.

(52) Overbeek, J. T. G.; Voorn, M. J. Phase Separation in Polyelectrolyte Solutions; Theory of Complex Coacervation. *J. Cell. Physiol. Suppl.* **1957**, *49* (S1), 7−26.

(53) Michaeli, I.; Overbeek, J. T. G.; Voorn, M. J. Phase Separation of Polyelectrolyte Solutions. *J. Polym. Sci.* **1957**, *23* (103), 443−450.

(54) Flory, P. J. *Principles of Polymer Chemistry*, Flory, P. J., Ed.; Cornell University Press, 1953.

(55) Ermoshkin, A. V.; Olvera De La Cruz, M. A Modified Random Phase Approximation of Polyelectrolyte Solutions. *Macromolecules* **2003**, *36* (20), 7824−7832.

(56) Kudlay, A.; Ermoshkin, A. V.; Olvera de la Cruz, M. Complexation of Oppositely Charged Polyelectrolytes: Effect of Ion Pair Formation. *Macromolecules* **2004**, *37* (24), 9231−9241.

(57) Kudlay, A.; Olvera de la Cruz, M. Precipitation of Oppositely Charged Polyelectrolytes in Salt Solutions. *J. Chem. Phys.* **2004**, *120* (1), 404−412.

(58) Ermoshkin, A. V.; Kudlay, A. N.; Olvera de la Cruz, M. Thermoreversible Crosslinking of Polyelectrolyte Chains. *J. Chem. Phys.* **2004**, *120* (24), 11930−11940.

(59) Olvera De La Cruz, M.; Belloni, L.; Delsanti, M.; Dalbiez, J. P.; Spalla, O.; Drifford, M. Precipitation of Highly Charged Polyelectrolyte Solutions in the Presence of Multivalent Salts. *J. Chem. Phys.* **1995**, *103* (13), 5781−5791.

(60) Borue, V. Y.; Erukhimovich, I. Y. A Statistical Theory of Globular Polyelectrolyte Complexes. *Macromolecules* **1990**, *23* (15), 3625−3632.

(61) Borue, V. Y.; Erukhimovich, I. Y. A Statistical Theory of Weakly Charged Polyelectrolytes: Fluctuations, Equation of State and Microphase Separation. *Macromolecules* **1988**, *21* (11), 3240−3249.

(62) Riggleman, R. A.; Kumar, R.; Fredrickson, G. H. Investigation of the Interfacial Tension of Complex Coacervates Using Field-Theoretic Simulations. *J. Chem. Phys.* **2012**, *136* (2), 024903.

(63) Popov, Y. O.; Lee, J.; Fredrickson, G. H. Field-Theoretic Simulations of Polyelectrolyte Complexation. *J. Polym. Sci., Part B: Polym. Phys.* **2007**, *45* (24), 3223−3230.

(64) Lee, J.; Popov, Y. O.; Fredrickson, G. H. Complex Coacervation: A Field Theoretic Simulation Study of Polyelectrolyte Complexation. *J. Chem. Phys.* **2008**, *128* (22), 224908.

(65) Kunze, K. K.; Netz, R. R. Morphologies of Semiflexible Polyelectrolyte Complexes. *Europhys. Lett.* **2002**, *58* (2), 299−305.

(66) de Vries, R.; Cohen Stuart, M. Theory and Simulations of Macroion Complexation. *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 295−301.

(67) Narambuena, C. F.; Leiva, E. P. M.; Chávez-Páez, M.; Pérez, E. Effect of Chain Stiffness on the Morphology of Polyelectrolyte Complexes. A Monte Carlo Simulation Study. *Polymer (Guildf)* **2010**, *51* (14), 3293−3302.

(68) Gus'kova, O. A.; Pavlov, A. S.; Khalatur, P. G. Complexes Based on Rigid-Chain Polyelectrolytes: Computer Simulation. *Polym. Sci. - Ser. A* **2006**, *48* (7), 763−770.

(69) Fossépré, M.; Tuvi-Arad, I.; Beljonne, D.; Richeter, S.; Clément, S.; Surin, M. Binding Mode Multiplicity and Multiscale Chirality in the Supramolecular Assembly of DNA and a π-Conjugated Polymer. *ChemPhysChem* **2020**, *21* (23), 2543−2552.

(70) Rubio-Magnieto, J.; Thomas, A.; Richeter, S.; Mehdi, A.; Dubois, P.; Lazzaroni, R.; Clément, S.; Surin, M. Chirality in DNA-π-Conjugated Polymer Supramolecular Structures: Insights into the Self-Assembly. *Chem. Commun.* **2013**, *49* (48), 5483−5485.

(71) Charlebois, I.; Gravel, C.; Arrad, N.; Boissinot, M.; Bergeron, M. G.; Leclerc, M. Impact of DNA Sequence and Oligonucleotide Length on a Polythiophene-Based Fluorescent DNA Biosensor. *Macromol. Biosci.* **2013**, *13* (6), 717−722.

(72) Dwight, S. J.; Gaylord, B. S.; Hong, J. W.; Bazan, G. C. Perturbation of Fluorescence by Nonspecific Interactions between Anionic Poly(Phenylenevinylene)s and Proteins: Implications for Biosensors. *J. Am. Chem. Soc.* **2004**, *126* (51), 16850−16859.

(73) Wang, D.; Gong, X.; Heeger, P. S.; Rininsland, F.; Bazan, G. C.; Heeger, A. J. Biosensors from Conjugated Polyelectrolyte Complexes. *Proc. Natl. Acad. Sci. U. S. A* **2002**, *99* (1), 49−53.

(74) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. DNA Detection Using Water-Soluble Conjugated Polymers and Peptide Nucleic Acid Probes. *Proc. Natl. Acad. Sci. U. S. A* **2002**, *99* (17), 10954−10957.

(75) Feng, F.; Tang, Y.; He, F.; Yu, M.; Duan, X.; Wang, S.; Li, Y.; Zhu, D. Cationic Conjugated Polymer/DNA Complexes for Amplified Fluorescence Assays of Nucleases and Methyltransferases. *Adv. Mater.* **2007**, *19* (21), 3490−3495.

(76) Feng, F.; Tang, Y.; Wang, S.; Li, Y.; Zhu, D. Continuous Fluorometric Assays for Acetylcholinesterase Activity and Inhibition with Conjugated Polyelectrolytes. *Angew. Chem.* **2007**, *119* (41), 8028−8032.

(77) He, F.; Tang, Y.; Yu, M.; Wang, S.; Li, Y.; Zhu, D. Fluorescence-Amplifying Detection of Hydrogen Peroxide with Cationic Conjugated Polymers, and Its Application to Glucose Sensing. *Adv. Funct. Mater.* **2006**, *16* (1), 91−94.

(78) An, L.; Wang, S.; Zhu, D. Conjugated Polyelectrolytes for Protein Assays and for the Manipulation of the Catalytic Activity of Enzymes. *Chem. - An Asian J.* **2008**, *3* (8−9), 1601−1606.

(79) Tang, Y.; Feng, F.; Yu, M.; An, L.; He, F.; Wang, S.; Li, Y.; Zhu, D.; Bazan, G. C. G. C. Direct Visualization of Glucose Phosphorylation with a Cationic Polythiophene. *Adv. Mater.* **2008**, *20* (4), 703−705.

(80) He, F.; Feng, F.; Wang, S.; Li, Y.; Zhu, D. Fluorescence Ratiometric Assays of Hydrogen Peroxide and Glucose in Serum Using Conjugated Polyelectrolytes. *J. Mater. Chem.* **2007**, *17* (35), 3702−3707.

(81) Vukmirović, N.; Wang, L. W. Electronic Structure of Disordered Conjugated Polymers: Polythiophenes. *J. Phys. Chem. B* **2009**, *113* (2), 409−415.

(82) Ding, H.; Pan, Z.; Pigani, L.; Seeber, R.; Zanardi, C. P- and n-Doping Processes in Polythiophene with Reduced Bandgap. An Electrochemical Impedance Spectroscopy Study. *Electrochim. Acta* **2001**, *46* (17), 2721−2732.

(83) Takechi, K.; Shiga, T.; Motohiro, T.; Akiyama, T.; Yamada, S.; Nakayama, H.; Kohama, K. Solar Cells Using Iodine-Doped Polythiophene-Porphyrin Polymer Films. *Sol. Energy Mater. Sol. Cells* **2006**, *90* (9), 1322−1330.

(84) Shakya, A.; Girard, M.; King, J. T.; Olvera de la Cruz, M. Role of Chain Flexibility in Asymmetric Polyelectrolyte Complexation in Salt Solutions. *Macromolecules* **2020**, *53* (4), 1258−1269.

(85) Li, Y.; Vamvounis, G.; Holdcroft, S. Tuning Optical Properties and Enhancing Solid-State Emission of Poly (Thiophene) s by Molecular Control : A Postfunctionalization Approach. *Macromolecules* **2002**, *35* (18), 6900−6906.

(86) Rajwar, D.; Ammanath, G.; Cheema, J. A.; Palaniappan, A.; Yildiz, U. H.; Liedberg, B. Tailoring Conformation-Induced Chromism of Polythiophene Copolymers for Nucleic Acid Assay at Resource Limited Settings. *ACS Appl. Mater. Interfaces* **2016**, *8* (13), 8349−8357.

(87) Ammanath, G.; Yeasmin, S.; Srinivasulu, Y.; Vats, M.; Cheema, J. A.; Nabilah, F.; Srivastava, R.; Yildiz, U. H.; Alagappan, P.; Liedberg, B. Flow-through Colorimetric Assay for Detection of Nucleic Acids in Plasma. *Anal. Chim. Acta* **2019**, *1066*, 102−111.

(88) Yucel, M.; Koc, A.; Ulgenalp, A.; Akkoc, G. D.; Ceyhan, M.; Yildiz, U. H. PCR-Free Methodology for Detection of Single-Nucleotide Polymorphism with a Cationic Polythiophene Reporter. *ACS Sensors* **2021**, *6* (3), 950−957.

(89) McCullough, R. D.; Ewbank, P. C.; Loewe, R. S. Self-Assembly and Disassembly of Regioregular, Water Soluble Polythiophenes: Chemoselective Ionchromatic Sensing in Water. *J. Am. Chem. Soc.* **1997**, *119* (3), 633−634.

(90) Kim; Chen, L.; Gong; Osada, Y.; et al. Titration Behavior and Spectral Transitions of Water-Soluble Polythiophene Carboxylic Acids. *Macromolecules* **1999**, *32* (12), 3964−3969.

(91) Yashima, E.; Maeda, K.; Okamoto, Y. Memory of Macromolecular Helicity Assisted by Interaction with Achiral Small Molecules. *Nature* **1999**, *399* (6735), 449−451.

(92) Maeda, K.; Okada, S.; Yashima, E.; Okamoto, Y. Helix Formation of Poly(Phenylacetylene) Derivatives Bearing Amino Groups at the Meta Position Induced by Optically Active Carboxylic Acids. *J. Polym. Sci. Part A Polym. Chem.* **2001**, *39* (18), 3180−3189.

(93) Ishikawa, M.; Maeda, K.; Yashima, E. Macromolecular Chirality Induction on Optically Inactive Poly(4-Carboxyphenyl Isocyanide) with Chiral Amines: A Dynamic Conformational Transition of Poly(Phenyl Isocyanide) Derivatives. *J. Am. Chem. Soc.* **2002**, *124* (25), 7448−7458.

(94) Yashima, E.; Matsushima, T.; Okamoto, Y. Chirality Assignment of Amines and Amino Alcohols Based on Circular Dichroism Induced by Helix Formation of a Stereoregular Poly((4-Carboxyphenyl)Acetylene) through Acid-Base Complexation. *J. Am. Chem. Soc.* **1997**, *119* (27), 6345−6359.

(95) Cieślar-Pobuda, A.; Bäck, M.; Magnusson, K.; Jain, M. V.; Rafat, M.; Ghavami, S.; Nilsson, K. P. R.; Los, M. J. Cell Type Related Differences in Staining with Pentameric Thiophene Derivatives. *Cytom. Part A* **2014**, *85* (7), 628−635.

(96) Magnusson, K.; Appelqvist, H.; Cieślar-Pobuda, A.; Wigenius, J.; Karlsson, T.; Los, M. J.; Kågedal, B.; Jonasson, J.; Nilsson, K. P. R. Differential Vital Staining of Normal Fibroblasts and Melanoma Cells by an Anionic Conjugated Polyelectrolyte. *Cytom. Part A* **2015**, *87* (3), 262−272.

(97) Lichon, L.; Kotras, C.; Myrzakhmetov, B.; Arnoux, P.; Daurat, M.; Nguyen, C.; Durand, D.; Bouchmella, K.; Ali, L. M. A.; Durand, J. O.; et al. Polythiophenes with Cationic Phosphonium Groups as Vectors for Imaging, SiRNA Delivery, and Photodynamic Therapy. *Nanomater. 2020, Vol. 10, Page 1432* **2020**, *10* (8), 1432.

(98) Chang, C.-T.; Chang, C.-J.; Lee, C.-T.; Lin, F.-L.; Tsai, J.-D.; Ashendel, C. L.; Chan, T. C. K.; Geahlen, R. L. Polythiophene Anti-Tumor Agents. International Patent WO1998023269A1.

(99) Sánchez-Jiménez, M.; Estrany, F.; Borràs, N.; Maiti, B.; Díaz Díaz, D.; Del Valle, L. J.; Alemán, C. Antimicrobial Activity of Poly(3,4-Ethylenedioxythiophene) n-Doped with a Pyridinium-Containing Polyelectrolyte. *Soft Matter* **2019**, *15* (38), 7695−7703.

(100) Scheberl, A.; Khalil, M. L.; Maghsoodi, F.; Strach, E. W.; Yang, J.; Chi, E. Y.; Schanze, K. S.; Reimhult, E.; Whitten, D. G. Quantitative Determination of Dark and Light-Activated Antimicrobial Activity of Poly(Phenylene Ethynylene), Polythiophene, and Oligo(Phenylene Ethynylene) Electrolytes. *ACS Appl. Mater. Interfaces* **2020**, *12* (19), 21322−21329.

(101) Brown, D. M.; Yang, J.; Strach, E. W.; Khalil, M. I.; Whitten, D. G. Size and Substitution Effect on Antimicrobial Activity of Polythiophene Polyelectrolyte Derivatives Under Photolysis and Dark Conditions. *Photochem. Photobiol.* **2018**, *94* (6), 1116−1123.

(102) Wang, H. J.; Chen, C. P.; Jeng, R. J. Polythiophenes Comprising Conjugated Pendants for Polymer Solar Cells: A Review. *Materials (Basel)* **2014**, *7* (4), 2411.

(103) Jia, X.; Chen, Z.; Duan, C.; Wang, Z.; Yin, Q.; Huang, F.; Cao, Y. Polythiophene Derivatives Compatible with Both Fullerene and Non-Fullerene Acceptors for Polymer Solar Cells. *J. Mater. Chem. C* **2019**, *7* (2), 314−323.

(104) Xiao, J.; Jia, X.; Duan, C.; Huang, F.; Yip, H. L.; Cao, Y. Surpassing 13% Efficiency for Polythiophene Organic Solar Cells Processed from Nonhalogenated Solvent. *Adv. Mater.* **2021**, *33* (25), 2008158.

(105) Mehmood, U.; Al-Ahmed, A.; Hussein, I. A. Review on Recent Advances in Polythiophene Based Photovoltaic Devices. *Renew. Sustain. Energy Rev.* **2016**, *57*, 550−561.

(106) Yan, W.; Jiang, D.; Liu, Q.; Kang, Q.; Zhou, F. Solar Cells Constructed with Polythiophene Thin Films Grown along Tethered Thiophene-Dye Conjugates via Photoelectrochemical Polymerization. *ACS Appl. Mater. Interfaces* **2019**, *11* (20), 18755−18762.

(107) Kuo, C. T.; Thompson, A. M.; Gallina, M. E.; Ye, F.; Johnson, E. S.; Sun, W.; Zhao, M.; Yu, J.; Wu, I. C.; Fujimoto, B.; et al. Optical Painting and Fluorescence Activated Sorting of Single Adherent Cells Labelled with Photoswitchable Pdots. *Nat. Commun.* **2016**, *7* (1), 1−11.

(108) Pu, K.; Mei, J.; Jokerst, J. V.; Hong, G.; Antaris, A. L.; Chattopadhyay, N.; Shuhendler, A. J.; Kurosawa, T.; Zhou, Y.; Gambhir, S. S.; et al. Diketopyrrolopyrrole-Based Semiconducting Polymer Nanoparticles for In Vivo Photoacoustic Imaging. *Adv. Mater.* **2015**, *27* (35), 5184−5190.

(109) Wu, C.; Bull, B.; Szymanski, C.; Christensen, K.; McNeill, J. Multicolor Conjugated Polymer Dots for Biological Fluorescence Imaging. *ACS Nano* **2008**, *2* (11), 2415−2423.

(110) Yang, Y.; Chen, J.; Yang, Y.; Xie, Z.; Song, L.; Zhang, P.; Liu, C.; Liu, J. A 1064 Nm Excitable Semiconducting Polymer Nanoparticle for Photoacoustic Imaging of Gliomas. *Nanoscale* **2019**, *11* (16), 7754−7760.

(111) Yu, J.; Rong, Y.; Kuo, C. T.; Zhou, X. H.; Chiu, D. T. Recent Advances in the Development of Highly Luminescent Semiconducting Polymer Dots and Nanoparticles for Biological Imaging and Medicine. *Anal. Chem.* **2017**, *89*, 42−56.

(112) Wu, C.; Schneider, T.; Zeigler, M.; Yu, J.; Schiro, P. G.; Burnham, D. R.; McNeill, J. D.; Chiu, D. T. Bioconjugation of Ultrabright Semiconducting Polymer Dots for Specific Cellular Targeting. *J. Am. Chem. Soc.* **2010**, *132* (43), 15410−15417.

(113) Tu, M. C.; Rajwar, D.; Ammanath, G.; Alagappan, P.; Yildiz, U. H.; Liedberg, B. Visual Detection of Al3+ Ions Using Conjugated Copolymer-ATP Supramolecular Complex. *Anal. Chim. Acta* **2016**, *912*, 105−110.

(114) Ho, H. A.; Leclerc, M. New Colorimetric and Fluorometric Chemosensor Based on a Cationic Polythiophene Derivative for Iodide-Specific Detection. *J. Am. Chem. Soc.* **2003**, *125* (15), 4412−4413.

(115) Wang, J.; Zhang, Q.; Tan, K. J.; Long, Y. F.; Ling, J.; Huang, C. Z. Observable Temperature-Dependent Compaction-Decompaction of Cationic Polythiophene in the Presence of Iodide. *J. Phys. Chem. B* **2011**, *115* (7), 1693−1697.

(116) Wang, X.; Zhang, C.; Zhang, Y.; Sun, J.; Cao, L.; Ji, J.; Feng, F. Facile Crosslinking of Polythiophenes by Polyethylenimine via Ester Aminolysis for Selective Cu(II) Detection in Water. *Biosens. Bioelectron.* **2018**, *109*, 255−262.

(117) Ghosh, R.; Das, S.; Chatterjee, D. P.; Nandi, A. K. Cationic Polythiophene for Specific Detection of Cyanide Ions in Water Using Fluorometric Technique. *RSC Adv.* **2015**, *5* (112), 92564−92572.

(118) Kohnken, R.; Kodigepalli, K. M.; Wu, L. Regulation of Deoxynucleotide Metabolism in Cancer: Novel Mechanisms and Therapeutic Implications. *Molecular Cancer* **2015**, *14*, 1.

(119) Knaapila, M.; Evans, R. C.; Garamus, V. M.; Almásy, L.; Székely, N. K.; Gutacker, A.; Scherf, U.; Burrows, H. D. Structure and "Surfactochromic" Properties of Conjugated Polyelectrolyte (CPE): Surfactant Complexes between a Cationic Polythiophene and SDS in Water. *Langmuir* **2010**, *26* (19), 15634−15643.

(120) Wang, L.; Feng, Q.; Wang, X.; Pei, M.; Zhang, G. A Novel Polythiophene Derivative as a Sensitive Colorimetric and Fluorescent Sensor for Anionic Surfactants in Water. *New J. Chem.* **2012**, *36* (9), 1897−1901.

(121) Li, E.; Lin, L.; Wang, L.; Pei, M.; Xu, J.; Zhang, G. Synthesis of a New Cationic Polythiophene Derivative and Its Application for Colorimetric and Fluorometric Detection of Iodide Ion and Anionic Surfactants in Water. *Macromol. Chem. Phys.* **2012**, *213* (9), 887−892.

(122) Evans, R. C.; Knaapila, M.; Willis-Fox, N.; Kraft, M.; Terry, A.; Burrows, H. D.; Scherf, U. Cationic Polythiophene-Surfactant Self-Assembly Complexes: Phase Transitions, Optical Response, and Sensing. *Langmuir* **2012**, *28* (33), 12348−12356.

(123) Ho, H.-A.; Boissinot, M.; Bergeron, M. G.; Corbeil, G.; Doré, K.; Boudreau, D.; Leclerc, M. Colorimetric and Fluorometric Detection of Nucleic Acids Using Cationic Polythiophene Derivatives. *Angew. Chem.* **2002**, *41* (9), 1548−1551.

(124) Ho, H. A.; Leclerc, M. Optical Sensors Based on Hybrid Aptamer/Conjugated Polymer Complexes. *J. Am. Chem. Soc.* **2004**, *126* (5), 1384−1387.

(125) Yao, Z.; Bai, H.; Li, C.; Shi, G. Colorimetric and Fluorescent Dual Probe Based on a Polythiophene Derivative for the Detection of Cysteine and Homocysteine. *Chem. Commun.* **2011**, *47* (26), 7431−7433.

(126) Maynor, M. S.; Nelson, T. L.; O'Sullivan, C.; Lavigne, J. J. A Food Freshness Sensor Using the Multistate Response from Analyte-Lnduced Aggregation of a Cross-Reactive Poly(Thiophene). *Org. Lett.* **2007**, *9* (17), 3217−3220.

(127) Applefield, D.; Krishnan, S. Protamine. In *StatPearls*; Treasure Island, FL, 2020.

(128) Refsum, H.; Ueland, P. M.; Nygard, O.; Vollset, S. E. Homocysteine and Cardiovascular Disease. *Annu. Rev. Med.* **1998**, *49* (1), 31−62.

(129) Shahrokhian, S. Lead Phthalocyanine as a Selective Carrier for Preparation of a Cysteine-Selective Electrode. *Anal. Chem.* **2001**, *73* (24), 5972−5978.

(130) Seshadri, S.; Beiser, A.; Selhub, J.; Jacques, P. F.; Rosenberg, I. H.; D'Agostino, R. B.; Wilson, P. W. F.; Wolf, P. A. Plasma Homocysteine as a Risk Factor for Dementia and Alzheimer's Disease. *N. Engl. J. Med.* **2002**, *346* (7), 476−483.

(131) Sinsinbar, G.; Gudlur, S.; Wood, S. E.; Ammanath, G.; Yildiz, H. U.; Alagappan, P.; Mrksich, M.; Liedberg, B. Outer Membrane Protease (OmpT)-based E. Coli Sensing with Anionic Polythiophene and Unlabeled Peptide Substrate. *Angew. Chem.* **2020**, *132*, 18224.

(132) Åslund, A.; Herland, A.; Hammarström, P.; Nilsson, K. P. R.; Jonsson, B. H.; Inganäs, O.; Konradsson, P. Studies of Luminescent Conjugated Polythiophene Derivatives: Enhanced Spectral Discrim-

ination of Protein Conformational States. *Bioconjugate Chem.* **2007**, *18* (6), 1860−1868.

(133) Åslund, A.; Sigurdson, C. J.; Klingstedt, T.; Grathwohl, S.; Bolmont, T.; Dickstein, D. L.; Glimsdal, E.; Prokop, S.; Lindgren, M.; Konradsson, P.; et al. Novel Pentameric Thiophene Derivatives for in Vitro and in Vivo Optical Imaging of a Plethora of Protein Aggregates in Cerebral Amyloidoses. *ACS Chem. Biol.* **2009**, *4* (8), 673−684.

(134) Sun, T.; Niu, Q.; Li, Y.; Li, T.; Hu, T.; Wang, E.; Liu, H. A Novel Oligothiophene-Based Colorimetric and Fluorescent "Turn on" Sensor for Highly Selective and Sensitive Detection of Cyanide in Aqueous Media and Its Practical Applications in Water and Food Samples. *Sensors Actuators, B Chem.* **2018**, *258*, 64−71.

(135) Kumar, G. R.; Sarkar, S. K.; Thilagar, P. Aggregation-Induced Emission and Sensing Characteristics of Triarylborane−Oligothiophene−Dicyanovinyl Triads. *Chem. - A Eur. J.* **2016**, *22* (48), 17215−17225.

(136) Magnusson, K.; Simon, R.; Sjölander, D.; Sigurdson, C. J.; Hammarström, P.; Nilsson, K. P. R. Multimodal Fluorescence Microscopy of Prion Strain Specific PrP Deposits Stained by Thiophene-Based Amyloid Ligands. *Prion* **2014**, *8* (4), 319−329.

(137) Magnusson, K.; Appelqvist, H.; Cieslar-Pobuda, A.; Bäck, M.; Kågedal, B.; Jonasson, J. A.; Los, M. J.; Nilsson, K. P. R. An Imidazole Functionalized Pentameric Thiophene Displays Different Staining Patterns in Normal and Malignant Cells. *Front. Chem.* **2015**, *3* (OCT), 1−11.

(138) Åslund, A.; Nilsson, K. P. R.; Konradsson, P. Fluorescent Oligo and Poly-Thiophenes and Their Utilization for Recording Biological Events of Diverse Origin-When Organic Chemistry Meets Biology. *J. Chem. Biol.* **2009**, *2* (4), 161−175.

(139) Klingstedt, T.; Blechschmidt, C.; Nogalska, A.; Prokop, S.; Häggqvist, B.; Danielsson, O.; Engel, W. K.; Askanas, V.; Heppner, F. L.; Nilsson, K. P. R. Luminescent Conjugated Oligothiophenes for Sensitive Fluorescent Assignment of Protein Inclusion Bodies. *ChemBioChem.* **2013**, *14* (5), 607−616.

(140) Klingstedt, T.; Åslund, A.; Simon, R. A.; Johansson, L. B. G.; Mason, J. J.; Nyström, S.; Hammarström, P.; Nilsson, K. P. R. Synthesis of a Library of Oligothiophenes and Their Utilization as Fluorescent Ligands for Spectral Assignment of Protein Aggregates. *Org. Biomol. Chem.* **2011**, *9* (24), 8356−8370.

(141) Klingstedt, T.; Shirani, H.; Åslund, K. O. A. A.; Cairns, N. J.; Sigurdson, C. J.; Goedert, M.; Nilsson, K. P. R. The Structural Basis for Optimal Performance of Oligothiophene-Based Fluorescent Amyloid Ligands: Conformational Flexibility Is Essential for Spectral Assignment of a Diversity of Protein Aggregates. *Chem. - A Eur. J.* **2013**, *19* (31), 10179−10192.

(142) Duarte, A.; Chworos, A.; Flagan, S. F.; Hanrahan, G.; Bazan, G. C. Identification of Bacteria by Conjugated Oligoelectrolyte/Single-Stranded DNA Electrostatic Complexes. *J. Am. Chem. Soc.* **2010**, *132* (36), 12562−12564.

(143) Tang, Y.; Achyuthan, K. E.; Whitten, D. G. Label-Free and Real-Time Sequence Specific DNA Detection Based on Supramolecular Self-Assembly. *Langmuir* **2010**, *26* (9), 6832−6837.

(144) Pu, K. Y.; Shi, J.; Wang, L.; Cai, L.; Wang, G.; Liu, B. Mannose-Substituted Conjugated Polyelectrolyte and Oligomer as an Intelligent Energy Transfer Pair for Label-Free Visual Detection of Concanavalin A. *Macromolecules* **2010**, *43* (23), 9690−9697.

(145) Wang, S.; Liu, B.; Gaylord, B. S.; Bazan, G. C. Size-Specific Interactions Between Single- and Double-Stranded Oligonucleotides and Cationic Water-Soluble Oligofluorenes. *Adv. Funct. Mater.* **2003**, *13* (6), 463−467.

(146) Rittmeyer, S. P.; Groß, A. Structural and Electronic Properties of Oligo- and Polythiophenes Modified by Substituents. *Beilstein J. Nanotechnol.* **2012**, *3* (1), 909−919.

(147) Yeasmin, S.; Ammanath, G.; Ali, Y.; Boehm, B. O.; Yildiz, U. H.; Palaniappan, A.; Liedberg, B. Colorimetric Urinalysis for On-Site Detection of Metabolic Biomarkers. *ACS Appl. Mater. Interfaces* **2020**, *12*, 31270.

(148) Aydın, H. B.; Cheema, J. A.; Ammanath, G.; Toklucu, C.; Yucel, M.; Özenler, S.; Palaniappan, A.; Liedberg, B.; Yildiz, U. H.

Pixelated Colorimetric Nucleic Acid Assay. *Talanta* **2020**, *209*, 120581.

(149) Moon, J. H.; MacLean, P.; McDaniel, W.; Hancock, L. F. Conjugated Polymer Nanoparticles for Biochemical Protein Kinase Assay. *Chem. Commun.* **2007**, No. 46, 4910−4912.

(150) Sun, K.; Tang, Y.; Li, Q.; Yin, S.; Qin, W.; Yu, J.; Chiu, D. T.; Liu, Y.; Yuan, Z.; Zhang, X.; et al. In Vivo Dynamic Monitoring of Small Molecules with Implantable Polymer-Dot Transducer. *ACS Nano* **2016**, *10* (7), 6769−6781.

(151) Hu, D.; Yu, J.; Padmanaban, G.; Ramakrishnan, S.; Barbara, P. F. Spatial Confinement of Exciton Transfer and the Role of Conformational Order in Organic Nanoparticles. *Nano Lett.* **2002**, *2* (10), 1121−1124.

(152) Eder, T.; Stangl, T.; Gmelch, M.; Remmerssen, K.; Laux, D.; Höger, S.; Lupton, J. M.; Vogelsang, J. Switching between H- and J-Type Electronic Coupling in Single Conjugated Polymer Aggregates. *Nat. Commun.* **2017**, *8* (1), 1.

(153) Feng, L.; Zhu, C.; Yuan, H.; Liu, L.; Lv, F.; Wang, S. Conjugated Polymer Nanoparticles: Preparation, Properties, Functionalization and Biological Applications. *Chem. Soc. Rev.* **2013**, *42* (16), 6620−6633.

(154) Tuncel, D.; Demir, H. V. Conjugated Polymer Nanoparticles. *Nanoscale* **2010**, *2* (4), 484−494.

(155) Grey, J. K.; Kim, D. Y.; Norris, B. C.; Miller, W. L.; Barbara, P. F. Size-Dependent Spectroscopic Properties of Conjugated Polymer Nanoparticles. *J. Phys. Chem. B* **2006**, *110* (51), 25568−25572.

(156) Yu, J.; Wu, C.; Zhang, X.; Ye, F.; Gallina, M. E.; Rong, Y.; Wu, I.-C.; Sun, W.; Chan, Y.-H.; Chiu, D. T. Stable Functionalization of Small Semiconducting Polymer Dots via Covalent Cross-Linking and Their Application for Specific Cellular Imaging. *Adv. Mater.* **2012**, *24* (26), 3498−3504.

(157) Sun, K.; Chen, H.; Wang, L.; Yin, S.; Wang, H.; Xu, G.; Chen, D.; Zhang, X.; Wu, C.; Qin, W. Size-Dependent Property and Cell Labeling of Semiconducting Polymer Dots. *ACS Appl. Mater. Interfaces* **2014**, *6* (13), 10802−10812.

(158) Wu, L.; Wu, I. C.; Dufort, C. C.; Carlson, M. A.; Wu, X.; Chen, L.; Kuo, C. T.; Qin, Y.; Yu, J.; Hingorani, S. R.; et al. Photostable Ratiometric Pdot Probe for in Vitro and in Vivo Imaging of Hypochlorous Acid. *J. Am. Chem. Soc.* **2017**, *139* (20), 6911−6918.

(159) Özenler, S.; Yucel, M.; Tüncel, Ö.; Kaya, H.; Özçelik, S.; Yildiz, U. H. Single Chain Cationic Polymer Dot as a Fluorescent Probe for Cell Imaging and Selective Determination of Hepatocellular Carcinoma Cells. *Anal. Chem.* **2019**, *91* (16), 10357−10360.

(160) Yu, J.; Rong, Y.; Kuo, C. T.; Zhou, X. H.; Chiu, D. T. Recent Advances in the Development of Highly Luminescent Semiconducting Polymer Dots and Nanoparticles for Biological Imaging and Medicine. *Anal. Chem.* **2017**, *89*, 42−56.

(161) Kuo, C. T.; Thompson, A. M.; Gallina, M. E.; Ye, F.; Johnson, E. S.; Sun, W.; Zhao, M.; Yu, J.; Wu, I. C.; Fujimoto, B.; et al. Optical Painting and Fluorescence Activated Sorting of Single Adherent Cells Labelled with Photoswitchable Pdots. *Nat. Commun.* **2016**, *7* (1), 1−11.

(162) Sun, K.; Tang, Y.; Li, Q.; Yin, S.; Qin, W.; Yu, J.; Chiu, D. T.; Liu, Y.; Yuan, Z.; Zhang, X.; et al. In Vivo Dynamic Monitoring of Small Molecules with Implantable Polymer-Dot Transducer. *ACS Nano* **2016**, *10* (7), 6769−6781.

(163) Ghorbani Zamani, F.; Moulahoum, H.; Ak, M.; Odaci Demirkol, D.; Timur, S. Current Trends in the Development of Conducting Polymers-Based Biosensors. *TrAC - Trends Anal. Chem.* **2019**, *118*, 264−276.