Naphthalimide-Based Fluorescent Polymers for Molecular Detection

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The beginning of the 21st century was marked by the intensive development of fiber-optic sensors. New functional materials with excellent sensory properties are required to design such sensors. Fluorescent probes for neutral and charged molecules are constantly developing. However, only a small part of the reported probes was successfully converted into functional sensing polymers and found real-world applications. A great challenge is to retain the sensing properties of a probe in a polymer matrix. The purpose of this review is to understand how properties of a probe are changed upon incorporation into a polymer and to reveal successful approaches. The review focuses on the use of the naphthalimide-based probes in the construction of sensing polymers. The literature overview is presented according to the nature of the guest molecules targeted for the quantitative detection: cations, anions, and small organic molecules.

1. Introduction

Since the beginning of the 21st century, the market and research field of optical fibre sensors has been intensively grown.[1–3] They have a great potential to be applied in industrial analysis,[4] biochemistry,[5,6] medicine,[7] and other areas.[8] However, further development is not possible without the creation of new sensor materials.[9] Fluorescent sensor materials and polymers are of high interest because of their versatility and simplicity to incorporate into sensor devices.[10,11] During the recent years much attention has been paid to the design and preparation of different architectures based on 1,8-naphthalimide.[12–17] A number of strategies have been developed to synthesize fluorescent functional materials involving naphthalimide derivatives.[13–18]

Naphthalimides have a pharmacological significance as they are core scaffolds for antitumor, anti-inflammatory, antidepressant, antipROTOzoal and antiviral agents, etc.[20] The naphthalimide ring can also intercalate with DNA and thus perturb the cellular events.[21–23] Also naphthalimide-based reductive fluorescent sensors can be used to detect hypoxia in cancer.[24] Naphthalimides and analogues can be applied as antitumor agents with the different mechanisms of action.[25]

4-Amino-1,8-naphthalimide containing probes were applied to target selectively different kinds of subcellular organelles, to visualize enzyme substrates and analyse the enzyme catalytic activity. Several hybrid sensors based on protein tags labelled with 4-amino-1,8-naphthalimide fluorophores have also been constructed.[26] Recent efforts in design, synthesis, and biological use of a series of new water-soluble conjugated oligomers and polymers with antibacterial and anticancer functions through regulating cellular functions and biological processes were summarized in the review by Wang and coworkers.[27]

There are several mechanisms that are responsible for the sensory properties of 1,8 naphthalimide-based materials: photo-induced electron transfer (PET),[28] intramolecular charge transfer (ICT), fluorescence resonance energy transfer (FRET)[29] and aggregation-induced emission quenching or enhancement (AIEQ/AIEE).[30] The reported functional materials can respond to a wide array of potential analytes such as protons,[31] metal cations,[32–36] anions,[37–39] organic,[40–42] and biomolecules,[43–46] water vapor,[47] and temperature.[48] The most widely exploited recognition moieties for these analytes are cyclam, calixarenes, cyclodextrins and crown ethers; however, other macrocyclic and non-macrocyclic receptors were also investigated.[49,50] Despite this progress in the creation of single-molecule sensors, examples of fluorescent polymer sensor materials utilizing...
1,8-naphthalimides as a fluorescent reporting unit for various cations and anions are still rare.[51–53] Considering the process of creating polymer-based sensory materials, it is necessary to mention several important properties of naphthalimide, which are already actively used to create polymers. The approaches for “one-step” coloration and stabilization of polymers by a combination “in-one molecule” of a chromophore and a stabilizer were demonstrated.[54] Recent advances on naphthalic anhydrides and 1,8-naphthalimide-based photoinitiators of polymerization were described in details by G. Noirbent and F. Dumur in their review.[55]

In this review, we have gathered the literature devoted to the creation of fluorescent sensor polymers based on naphthalimides, where the dye derivative is covalently bound to a polymer matrix. We focus on the following questions during the literature analysis:

1. What is the relationship between the structure of naphthalimide-based probes and their binding and sensing properties toward anion, cations and neutral molecules?
2. What are the spectroscopic properties of molecular probes and their behaviour in the presence of an analyte?
3. What are the approaches toward sensing polymer synthesis based on naphthalimide-containing probes?
4. How the sensing properties of a polymer differ from those of the related molecular probe, especially by interacting with aqueous solution of an analyte?
5. What are the strategies to retain or improve the sensing properties of a probe in the polymer matrix?

The review is divided into several parts according to the nature of the guest molecules targeted for the quantitative detection: cations, anions and small organic molecules. As far as we know, this review is the first attempt to summarize publications related to the topic of fluorescent sensory polymers based on naphthalimide dyes.

2. Sensors for Cations

2.1. pH-Sensors

Functional polymers that are able to detect pH attract considerable attention because of their potential application in biology. A series of sensing polymers were prepared, in which naphthalimide derivatives were copolymerized with different monomers such as methyl methacrylate, styrene, 2-hydroxyethyl methacrylate,[56] dimethylbicyclo[2.2.1] hept-5-ene-2,3-dicarboxylate[57] and acrylamide. Alkylpiperazine derivative of naphthalimides belong to the most popular structures, since they show best sensitivity, even in the complex media.

For instance, Tian group synthesized a polymer using RAFT polymerization method containing naphthalimide-piperazine conjugate. They have studied methylated and quaternized piperazine derivatives. The polymers showed “Off-On-Off” behavior, where the polymer film has better sensitivity than the monomer naphthalimide derivative.[58]

Catalina and co-workers synthesized polymer membranes, to which they attached piperazine naphthalimides through different positions (Figure 1) and studied the resulting polymers for the pH sensing ability.[59] The polymer membrane was prepared by photocrosslinking of vinyl-pyrrolidone/butyl acrylate and ethylene glycol dimethacrylate, together with a reactive monomer—methacryloyl chloride, which was later reacted with naphthalimide dye. This combination of hydrophobic and hydrophilic groups provides a versatility of the future applications of the membranes. The polymers showed excellent pH sensitivity in the pH range 4.0–9.0. The response time of the polymers was in most cases less than 15 min, however it was dependent on the thickness of the membrane. Similar study was carried out by the Meldrum group, in which the authors also varied the position of the attachment of the naphthalimide-piperazine-metacrylate conjugates.[60] The monomers were successfully used as new intracellular sensors, while polymer films were utilized as sensors for the extracellular pH determination. The polymer sensors have less sensitivity to pH, likely because of the interaction with the polymer chain. The attachment of the naphthalimide through the amide moiety resulted in the film with best sensing properties.

2.2. Transition Metal Ion Sensors

Metal ions play essential roles in the industrial and biological processes. However, the unregulated amounts may cause
ecological problems and be harmful for people. During the last decade the designs and syntheses of fluorescent and colorimetric chemosensors with high selectivity and sensitivity for the detection of metal ions in the environmental and biological systems have been developed.[61,62] Chemosensors based on naphthalimides were constructed for the monitoring of various metal ions.[83] In addition to polymeric materials, naphthalimide sensors can be attached to a silicone substrate.[64,65] Silica-based optical chemosensors with the attached naphthalimide fluorescent sensors for Hg²⁺, Cu²⁺, Cd²⁺, Pb²⁺ have found practical application in ion detection as reviewed by Yan and coworkers.[66]

Pioneering work on the synthesis of fluorescent polymer sensors for transition metal cations using naphthalimides were the publications of Grabchev and co-workers.[67–69] For instance, compound 1 served as a starting point for studying the properties of the derived materials. Authors proposed that the fluorophore should show strong PET and produce strong changes in fluorescence upon protonation or coordination of transition metal cations. This should happen only if fluorophores are bound to the polymer chain through covalent bonds regularly with a certain distance between each other in order to avoid intramolecular interactions. With this idea in mind two yellow-green fluorescent co-polymers of 1 with methyl methacrylate (MMA) and styrene (ST) have been obtained and their protonation behaviour was tested.

In the resulted polymers P1-MMA (P1 designates the derived polymer based on monomer 1) and P1-St, the naphthalimide derivative is covalently attached to the polymer chain with 0.94 and 0.86 wt %, respectively. The molecular weight and molecular weight distribution $M_n/M_w$ of these polymers suggest the formation of high molecular weight polymers: $M_n = 1.21 \times 10^5$ (g mol⁻¹) and $M_w = 0.64 \times 10^5$ for P1-St and $M_n = 0.98 \times 10^5$ and $M_w = 0.57 \times 10^5$ for P1-MMA. The authors studied spectral properties of materials in solution and in the solid state (films). Interestingly, the fluorescence spectra of naphthalimide derivative 1 and copolymers in chloroform appeared to be identical. The fluorescence of the solid film was hypsochromically shifted as compared to the polymers in a dissolved state. The results showed that the polymeric matrix and/or to the co-polymerization process could indeed alter the spectroscopic properties of the naphthalimide derivative.

The ratio of fluorescence intensity at pH 3.5 and pH 10.5 of aqueous solutions of monomer 1 and co-polymers P1-MMA and P1-St gave FE (fluorescence enhancement $F/F_0$, $F$-intensity, $F_0$-initial intensity at basic conditions) 18, 1.2, 1.9, respectively. The observed fluorescence enhancement is a result of the protonation event of the dimethylamino group, which blocks the PET process. The weak fluorescence enhancement (20%) for the polymers was explained by the formation of intermolecular interactions between the protonated amine receptor of the naphthalimide and the poly(methyl methacrylate) carbonyl groups, which weakens the inhibition of the PET from the receptor to the fluorophore (Figure 2).

The addition of the Cu²⁺ ions to the naphthalimide derivative solution up to concentration of $1.25 \times 10^{-3}$ M resulted in an increase of the fluorescence intensity ($FE = 4.4$), which corresponds to the formation of the intramolecular bidentate complex with Cu²⁺ ions. Above this concentration, the fluorescence intensity decreases due to the prevailing quenching interaction between the copper (II) ion and the fluorophore. Addition of Cu²⁺ ions to P1-MMA leads to a fluorescence increase ($FE = 2.2$). However, the same reaction with P1-St leads to fluorescent quenching ($FE = 0.62$). This interesting contrasting behaviour was explained by micro-structure of P1-St, which has higher rigidity and density of the macromolecules. Thus, Cu²⁺ ions were unable to form intramolecular bidentate complexes and were probably coordinated to the carbonyl groups of the naphthalimide derivative.

Knowing that fluorescence intensity depends strongly on the polarity of the solvents, authors studied the monomer and the copolymer in DMF.[68] Addition of different metal cations led to an increase of the fluorescence intensity of the monomer with the maximum $FE = 32.5$ for Fe²⁺, 8.91 for Ni²⁺, 11.25 for Zn²⁺, and 12.26 for Pb²⁺. On the one hand, the coordination of metal cations, inhibited PET process from the amine group to the fluorophore, which resulted in a fluorescence enhancement. On the other hand, electron-donating effect of the amine group in the 4th position was weakened leading to a hypsochromic shift. The pendant 4-N,N-dimethylaminoethyl-1,8-naphthalimide copolymer demonstrated the same PET, which is hindered in the presence of metal ions. It was shown that the addition of Fe³⁺ in the concentration of $10^{-6}$ M causes a significant increases of fluorescence intensity of DMF solution of the polymer ($FE = 8.9$) while other metal ions have FE in the range between 1.5 and 1.9.

An additional study of styrene copolymer P1-St revealed interesting properties in aqueous media.[70] The thin film (40 µm thick) was placed diagnostically into the sample quartz cuvette to improve the reproducibility of the spectroscopic experiments. The measurements were carried out in an acetic buffer aqueous solution at pH = 5 containing metal cations at concentrations of $0.1 \times 10^{-3}$ M. The rigid polymer structure of the P1-St film perturbs the diffusion of the metal cations inside the polymer. Therefore, the fluorescence emission in the presence of metal cations was investigated for a period of 20 min. Addition of Pb²⁺, Ni²⁺, Zn²⁺ led to a small decrease of fluorescence intensity (3% decrease), while Fe³⁺ quenched the fluorescence (35% decrease). The authors explained the selectivity by a strong competitive diffusion of Fe³⁺ into the polymer matrix.

Continuing research in this direction, the same group synthesized compound 2 and the copolymers with styrene P2-St and methylmethacrylate P2-MMA (Figure 2).[71] The results show that the amount of the monomeric 1,8-naphthalimide derivative bound to P2-St was 1.14 wt %, while for P2-MMA it was 1.25 wt %. The influence of metal cations on fluorescence intensity of the monomer was studied in acetonitrile. The strongest effect was observed in case of Cu²⁺ with $FE = 45.6$, which is two times stronger than the FE in the presence of Zn²⁺ and Fe³⁺. In all cases the emission band was shifted from 517 to 507 nm, indicating that the electron donating nitrogen at 4th position of the 1,8-naphthalimide participates in complexation with metal cations (conditions: acetic buffer pH = 5, metal cations concentration at $0.1 \times 10^{-3}$ M).

The largest optical response for metal cations was observed for the P2-St film. It was found that in case of Cu²⁺ and Zn²⁺ the binding effect on the fluorescence intensity was negligible.
(up to 2–4%) while Fe$^{3+}$ quenched the fluorescence with 45% decrease in intensity. This fact confirms that Fe$^{3+}$ do not form a complex with the amine group of the receptor, rather it is involved in the coordination of the carbonyl groups of the naphthalimide derivative.

Amino-functionalized naphthalimides 3 and 4 bearing styrene and the corresponding polymers were also studied in terms of Fe$^{3+}$ sensing in CH$_3$CN solution (Figure 2). The results of cation binding studies showed that naphthalimide derivatives were covalently attached to the polymer chain producing polymers P3-St and P4-St with 1.53 wt% and 1.45 wt%, respectively. While Fe$^{3+}$ showed FE = 16.4 in the presence of 3, the influence of the other cations under study (Mg$^{2+}$ > Co$^{2+}$ > Sr$^{2+}$ > Ag$^+$ > Cu$^{2+}$) on the fluorescence intensity of the monomer was negligible, except for Pb$^{2+}$, which showed FE = 9.1. Receptor 4 demonstrated much lower enhancement with all metal cations (maximum changes were FE = 3.7). The presence of the metal cations (Cu$^{2+}$, Sr$^{2+}$, Co$^{2+}$, Ni$^{2+}$, and Fe$^{3+}$) in aqueous solutions was studied with P3-St and P4-St films, having similar structure as P2-St, but containing compounds 3 and 4. In both cases the results were similar. The effect of most metal cations on the fluorescence intensity was insignificant –1–5% changes in fluorescence intensity. However, Fe$^{3+}$ resulted in almost completely quenched fluorescence (96%).

Xu et al reported a different approach, which involved the fabrication of a naphthalimide derivative on the quartz glass surface. Compound 5 with a terminal double bond was copolymerized with HEMA (2-hydroxyethyl methacrylate) under UV irradiation and covalently immobilized to the surface (Figure 3a).

Sensor P5-G shows a linear response toward Fe$^{3+}$ in the concentration range from $1.0 \times 10^{-5}$ to $1.0 \times 10^{-3}$ M and with detection limit of $4.5 \times 10^{-6}$ M (pH range 5–8). Addition of Fe$^{3+}$ into the Tris–HCl buffer (pH 6.02) causes 98% quenching of optode membrane fluorescence, while other cations showed almost no effect. Authors suggested that Fe$^{3+}$ coordinates to the carbonyl groups of the 1,8-naphthalimide, which leads to either an electron or energy transfer. The thickness of the optode membrane of 50 µm appeared to be optimal for the response time of the sensor, which was 100 and 150 s for $6.0 \times 10^{-4}$ and $1.0 \times 10^{-3}$ M Fe$^{3+}$ solutions, respectively. According to repeating measurements, the relative standard deviations from eleven measurements of blank buffer solution was 1.22%. A newly prepared membrane maintained its function for at least 2 months of continuous use.

An interesting example of a dual sensors was reported by Magri and coworkers, who immobilised a “Pourbaix sensor” onto a polymeric solid support and could detect concentration of both protons and Fe$^{3+}$ in water (Figure 3b). Thus,
compound 6 was covalently bound to the TentaGel. Upon addition of an acid and $20 \times 10^{-3}$ M Fe$^{3+}$ the authors observed the appearance of a green fluorescence. The fluorescent emission spectrum of compound 6 reveals ππ* ICT excited states with large Stokes shifts of ca. 130 nm resulting from the twisted conformation of the 4-amino substituents. A green fluorescence was also detected upon protonation of the alkylpiperazine and oxidation of ferrocene. These processes were the result of the simultaneous inhibition of ICT and PET processes.

While P6-R was designed with an “electron-donor–spacer$_1$–fluorophore–spacer$_2$–receptor–linker–bead” format, next sensor P7-R was synthesized with the module units and

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Figure 3. a) Schematic diagram describing the copolymerization (surface immobilization) of compound 5 and the possible binding mode for the optode membrane with Fe$^{3+}$. b) Tagged beads P6-R and P7-R and the design strategies for the covalently attached “Pourbaix sensors” 6 and 7 onto a polymer bead with colour-coded modules. TentaGels P6-R in c) MeOH under room lighting, d) under room lighting viewed through a digital camera and e) in MeOH irradiated with 365 nm light from a handheld UV lamp. Images of TentaGels P6-R on a glass microscope slide in f) “off” state in presence of Fe$^{3+}$, g) “off” state in presence of H$^+$, h) “on” state in presence of Fe$^{3+}$ and H$^+$. Conditions: $200 \times 10^{-3}$ M H$^+$ and $20 \times 10^{-3}$ M Fe$^{3+}$. Reproduced with permission.[74] Copyright 2018, The Royal Society of Chemistry.
connected in an alternative arrangement according to a “bead–linker–fluorophore–spacer_1–receptor–spacer_2–electron-donor” format (Figure 3b).[75] For compound 7, two quenching mechanisms are possible: ICT, which is provided by the piperazine unit and PET from ferrocene. The “off” states were attributed to the PET and ICT processes. In the presence of an acid and 50 × 10−6 m Fe3+ a green fluorescent was observed for both materials P6-R and P7-R. Addition of an aliquot of tetramethylammonium hydroxide (TMAH) deprotonates the receptor enabling the ICT process. Unfortunately, polymer beads did not form a suspension on agitation and readily settle to the bottom of the vial or cuvette. Therefore, photophysical parameters of functional materials were difficult to measure in standard quartz cuvettes.

Copolymer P8-Rh containing the rhodamine 6G spirolactam and 1,8-naphthalimide moieties was synthesized by using RAFT radical polymerization (Figure 4a).[76] The linear relationship between analytical answer and pH, the concentration of Fe3+ ion or temperature makes the copolymer as a multifunctional ratiometric fluorescent chemosensor. The fluorescent intensity at 555 nm relative to that at 520 nm (I555/I520) is dependent on the resonance energy transfer from 1,8-naphthalimide to rhodamine 6G. Interestingly, the coordination of Fe3+ to rhodamine 6G induces the spirolactam ring-opening resulting in fluorescence changes, while the coordination of Fe3+ to the piperazinyl moiety does not inhibit the PET process in the 1,8-naphthalimide moiety. As a result, the fluorescent intensity at 520 nm is not affected by Fe3+ and the emission at 555-nm of rhodamine 6G is significantly increased with the increasing concentration of Fe3+. The authors suggested that the FRET process occurs as the copolymer is excited at 400 nm.

A polythiophene-based conjugated polymer P9-T bearing 1,8-naphthalimide based pendants was prepared recently by a two-step modification of regioregular poly(3-(6-bromohexyl) thiophene) (Figure 4b).[77] The absorption and emission spectra of compound 8 showed significant changes only in the presence of Fe3+ among other studied metal ions (Co2+, Ni2+, Cu2+, Zn2+, Cd2+, Ag+, Hg2+, and Pb2+) were investigated in a buffered (Tris–HCl, pH 7.24) aqueous solution. The fluorescence studies revealed that the optode membrane shows strongest quenching in the presence of Cu2+ ions. As compared to the sensing properties of the parent compound 11, the optode membrane showed much better selectivity for Cu2+.

The strong binding affinity and a selective response of the obtained functional material was explained by a possible coordination of Cu2+ with the nitrogen atom of the 2-picolyl group. The competition experiments were carried out with P11-G in a buffered solution (Tris–HCl, pH 7.24) and in the presence of other metal ions. The response time of the sensor was 180 and 240 s for 4 × 10−5 and 4 × 10−4 M Cu2+ solutions and recovering time was independent on the Cu2+ concentration. As inferred from the detailed studies, the lifetime of the optode membrane was at least two months of continuous use. The P11-G sensor was used for the determination of Cu2+ in real samples: tap and river water and show satisfactory results.

Another chelating subunit—cyclam—was explored in nanoparticle-based FRET system P12-NP for the detection of Cu2+ in water. Fluorescent naphthalimide derivatives 12a, b and vinylbenzylcyclam (VBC) were covalently attached to the particles as shown in Figure 5a.[82] In the work, samples with increasing amounts of 12 in nanoparticles were investigated to determine the relationship between the fluophore concentration and the fluorescence intensity of the nanoparticle. By increasing the concentration of naphthalimide derivatives in the particles, the fluorescence intensity first increased and then slightly decreased, likely due to the self-quenching mechanism. Interestingly, P12a-NP exhibits much higher quenching capability in the presence of Cu2+ as compared to that of P12b-NP at the same naphthalimide derivative concentration. This phenomenon occurred due to the higher overlap of the donor’s emission spectrum 12a (455–650 nm) and the acceptor’s absorption spectrum VBC (Cu2+-cyclam complex 455–700 nm), thus providing more efficient FRET. In order to understand the high selectivity of nanoparticles for Cu2+, the fluorescence of the
dispersion was measured in the presence of competing metal cations. It was found that cations such as Co\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\), Hg\(^{2+}\), Mn\(^{2+}\), Ca\(^{2+}\), Mg\(^{2+}\), Fe\(^{2+}\), Pb\(^{2+}\) do not affect the emission. However, the addition of Cu\(^{2+}\) resulted in an effective fluorescence quenching in the pH range 4–10 (limit of detection (LOD): 5 \times 10^7 \text{ M}). The long-term photostability of nanoparticle

**Figure 4.** a) Schematic illustrations of the multistimulus responsive fluorescence behaviour of the copolymer P8-Rh in acidic conditions and Fe\(^{3+}\) aqueous solutions at room temperature. b) Synthetic route to the polymer P9-T. c) Structure of the Hybrane P1000 hyperbranched polymer and its modification to P10-H.
Mercury is one of the most popular sensing targets due to its toxicity. The group of Ma created a series of polymer sensors on a glass substrate able to detect mercury ions in aqueous solution. In their work, monomer 13 with a terminal double bond was copolymerized with HEMA by UV photopolymerization and covalently immobilized on the surface of the modified quartz glass plate (P13-G). The fluorescence responses of the optode membrane to various cations was studied by fixing the Hg\textsuperscript{2+} concentration at 1 × 10^{-4} M (pH 7.01, Tris–HCl buffer 50 × 10^{-3} M). The fluorescence intensity at 520 nm was enhanced after addition of Hg\textsuperscript{2+}. Other metal ions such as Fe\textsuperscript{3+}, Ni\textsuperscript{2+} and Cu\textsuperscript{2+} showed much lower enhancement (Figure 6).

Coordination of Hg\textsuperscript{2+} with the amine leads to hindering of the PET process resulting in a fluorescence enhancement of the functional material. P13-G shows a linear response toward Hg\textsuperscript{2+} in the concentration range from 6.0 × 10^{-6} to 2.0 × 10^{-4} M with LOD of 2.0 × 10^{-6} M and working pH range 4.0-7.5. The association constant for Hg\textsuperscript{2+} in 0.05 M Tris–HCl buffer solution (pH 7.01) was calculated as high as 3.5 × 10^{3} M\textsuperscript{-1}. The sensor is reversible and can be regenerated by washing with 0.01 M HCl and blank buffer solution. The response time of the optode membrane depends on the thickness of the membrane and concentration of the analyte. For example, 50 µm membrane has a response time of 50–80 s, while for recovering the materials one requires about 120 s. The proposed sensor P13-G was tested by the authors for the determination of Hg\textsuperscript{2+} in river water samples providing satisfactory results.

The Catalina group synthesized a mercury ion sensor, which is less sensitive to iron and copper ions. A selective fluorescence sensor P14-M was developed by grafting naphthalimide derivative 14 to a photocrosslinked membrane reaction through the acid chloride groups. The resulting fluorescence sensor demonstrated 3-fold fluorescence enhancement upon Hg\textsuperscript{2+} binding in pure water at pH 5.5–8.0 (Figure 6). The selectivity for Hg\textsuperscript{2+} was explained by the coordination of the metal cation to the (benzimidazolyl)methyl-piperazine subunit, which in turn prohibits the PET process from the piperazine to naphthalimide. The association constants $K_a$ and LOD of the 14 and P14-M complexes were 9657 M\textsuperscript{-1} (LOD 7.3 × 10^{-8} M) and 3627 M\textsuperscript{-1}, (2.5 × 10^{-6} M), respectively. The polymeric membrane could be reverted to the original state by washing with HCl solution. In this work the authors also confirmed the fact that the thickness of the membrane correlates with the response time. The thinner the membrane, the smaller was the response time.

### 2.3. Alkali and Alkaline Earth Metals Ion Sensors

The use of naphthalimide -functionalized crown ethers and related structures has been proven as a good approach for a selective cation detection.[28,85] For example, potassium selective PET ion sensor 15 was polymerized with 2-hydroxyethyl methacrylate (HEMA) and acrylamide (AM) to form a series of sensing films P15-G(1–8) (Figure 7).

The changes in fluorescence of the films toward potassium ions was improved through the adjustment of the HEMA and AM portions in the polymer, as well as through the introduction of positive or negative charge-containing segments. Interaction of 15 with potassium chloride (KCl) in 10 × 10^{-3} M HEPES buffer (pH 7.4) showed good selectivity over other metal cations at their physiological concentrations. FE factor for K\textsuperscript{+} was determined as 1.8, while other cations demonstrated insignificant changes in fluorescence with FE < 0.1. The sensitivity of the film P15-G4 (PHEMA-co-PAM with 80:15 ratio by weight) was better than that of pure 15. This improvement might be due to the fact that the sensing film is swollen by water, resulting in a better interaction of the receptor with K\textsuperscript{+}. Nevertheless, the association constants of 15 and P15-G4 were approximately
equal 133M$^{-1}$ ($K_d = 7.53 \times 10^{-3}$ m) and 138 m$^{-1}$ ($K_d = 7.25$), respectively. Properties of various sensing membranes (thickness 25 $\mu$m) are given in Table 1. The membrane P15-G1 made of PAM matrix did not respond to K$^+$ due to its poor permeability (Table 1). Films based on membranes P15-G3 and P15-G4 showed much better sensitivity than P15-G2, indicating that the combination PHEMA-co-PAM improves K$^+$ permeability. Increasing the density of negative charge on the film by using MESA (2-(methacryloyloxy)ethylsulfonic acid sodium salt) improved the sensitivity of the material for K$^+$. However, an increase in positive charge by using METAC (2-(methacryloyloxy)ethyltrimethyl ammonium chloride) decreased the sensitivity. Both free receptor and sensing film P15-G4 were successfully used as extracellular K$^+$-selective probe for bacteria species. Overall, the results of these studies confirm that the optimally selected composition of the polymer film can significantly increase the sensitivity and binding constants of the sensor.

Another approach to produce sensing materials by using fluorescent crown ethers utilizes receptor 16. The receptor was polymerized with N,N-dimethylacrylamide (Figure 8) forming a gel.$^{[87,88]}$

The effect of metal cations on the optical properties of receptor 16 and P16-HG was studied in acetonitrile. It was found that Ca$^{2+}$ (FE = 3), and Ba$^{2+}$ (FE = 9.4) cations induced the strongest increase in fluorescence, while no optical changes was observed for other metal cations.$^{[89]}$ A fluorescence increase was also observed for polymer P16-HG but to a slightly lower degree: Ca$^{2+}$ (FE = 2.3) and Ba$^{2+}$ (FE = 4.8). The difference in the sensing properties of monomer 16 and the gel was explained by the competition of dimethylcarbamido groups with crown ether for the formation of coordination with cations and coulomb repulsion of positively charged metal cations. Once the

Figure 6. Absorption and fluorescence spectra of 14 (A,C) at a concentration of $10^{-5}$ m in water:ethanol 4:1 (v/v) and for P14-M (B,D) containing $1.6 \times 10^{-4}$ m of bonded naphthalimide in buffered water ($pH = 7.0$) in the presence of increasing concentrations of Hg$^{2+}$ 0–3 equiv. E) Fluorescence emission intensity change with $-\log[Hg^{2+}]$. F) Job’s plot showing the stoichiometry of 14–Hg complex in buffered water. G) Structure of the polymer P13-G H) Proposed reversible binding mode P14-M with Hg$^{2+}$. Reproduced with permission.$^{[84]}$ Copyright 2018, Elsevier.
critical positive charge is reached, the polymer is not able to
bind cations anymore and the remaining portion of ionophoric
groups don’t participate in complexation.

A number of Ca^{2+}-selective probes has been patented. The
majority of the reported ionophores can bind the cation with
stability constants log K = 3.6. The approach to attach an iono-
phore to the surface was suggested by H. He and M. Mortel-
laro. Thus, receptor 17 containing a naphthalimide derivative
functioned as a PET sensor for Ca^{2+} ions. The receptor was
attached to activated cellulose using by N-hydroxysuccinimide
in DMF. The obtained aminocellulose fibres with immobilized
17 were suspended in 10% hydrogel in 90% ethanol-water and
then coated onto a polyester foil with a final dry thickness of
10 µm. The obtained film P17-C demonstrated the strongest
fluorescence increase in the presence of Ca^{2+}.

Similar strategy was used to design sensing materials for the
potassium (P15-C) and sodium ions P18-C (Figure 9).

The dynamic response of the sensor disk to different concen-
trations of sodium ions was studied at pH 7.4 in HEPES buffer.

The response time was less than 1 min and signal change was

Table 1. Compositions of the films P15-G1 to P15-G8, their sensitivity, and K_d values.

| Films  | Polymer compositions and their weight ratios | Sensitivity (F/F_0) | K_d [× 10^{-3} M] |
|--------|---------------------------------------------|--------------------|-------------------|
| P15-G1 | PAM                                         | 1.02 (±0.01)       | N/A               |
| P15-G2 | PHEMA                                       | 1.77 (±0.03)       | 18.5              |
| P15-G3 | PHEMA:PAM (50:40)                           | 2.03 (±0.03)       | 14.9              |
| P15-G4 | PHEMA:PAM (80:15)                           | 2.34 (±0.02)       | 7.25              |
| P15-G5 | PHEMA:PAM:PMETAC (80:15:20)                 | 1.59 (±0.02)       | 26.3              |
| P15-G6 | PHEMA:PAM:PMETAC (80:15:5)                  | 1.90 (±0.02)       | 15.8              |
| P15-G7 | PHEMA:PAM:PMESA (80:15:5)                   | 2.69 (±0.03)       | 6.04              |
| P15-G8 | PHEMA:PAM:PMESA (80:15:20)                  | 3.03 (±0.03)       | 4.96              |

a) Each film contains a 5%-weight ethoxylated trimethylolpropane triacrylate crosslinker; b) The sensitivity of the films to K^+ was measured at 0 and 10 × 10^{-3} M KCl according to extracellular K^+ concentration. F is the enhanced fluorescence intensity, F_0 the initial intensity.
detectable (0.5%/10⁻³ m) per millimolar change in sodium ion concentration. Interacting with sodium ions, the azacrown moiety turned orthogonal to the phenyl ring and directed toward the o-methoxy substituent participating in binding. The resulting conformation of the C=N bond decouples the nitrogen lone pair from the aromatic ring and disrupts the PET process with the same degree as the protonation of nitrogen. It was found that the ion-induced FE values for sodium and lithium were similar. The sensor films maintain their sensing properties and were stable against hydrolysis and oxidation, leading to slope changes of less than 5% after 9 months wet storage at 30°C.

The optodes for sodium, potassium and calcium were proposed as optical chemosensors for blood analysis. A special design was used to construct the sensors. Each of the three probes bear linkers for covalent immobilization to hydrophilic polymers and the attached on top of a transparent polyester foil covered with a black hydrophilic overcoat. The sensing and overcoat layers are hydrogels, which allow the diffusion of ions through the sensor, while larger species are blocked on the surface. According to the measurements, sensors bind Na⁺, K⁺, and Ca²⁺ reversibly with dissociation constants of 119 × 10⁻³, 17 × 10⁻³, 1.09 × 10⁻³ m, respectively.

Figure 8. a) Structure of receptor 16 and P16-HG. Fluorescence enhancement F/F₀ observed in acetonitrile solution of b) receptor 16 and c) P16-HG.

Figure 9. Structures of fluoroionophores for sodium, potassium and calcium.
3. Sensors for Anions

Despite the fact that the development of sensors for anions is usually a more difficult task than that for cations, significant progress in this direction has been made in recent years.\(^94\)\(^95\) Polymer based anion sensors is still an emerging field\(^96\) and only a few successful conjugations of anion receptors with a polymer matrix have been reported. These examples can be separated into two groups: sensors for fluoride and sensors for reactive anions such as hypochlorite.

Fluoride ions are involved in various physiological activities, such as water fluoridation, bone disease treatment and caries.\(^97\) Contamination with fluoride ions can cause serious risk to public health.\(^98\) Basic nature of the fluoride anion, small ionic radius with high charge density helped to develop several selective sensing materials.\(^99\)

An interesting example of fluoride selective sensor P19-F was synthesized by using RAFT polymerization (Figure 10a).\(^100\) The reversible addition-fragmentation chain transfer (RAFT) polymerization allowed Tian and co-workers to control the narrow molecular weight distribution and high purity of the product.

The effect of halide anions on the absorption and fluorescence of polymer film (thickness 30 µm) P19-F and free compound 19 was investigated in the mixture of CH\(_2\)Cl\(_2\)-DMSO (9:1 vol.). The fluorescence intensity of the P19-F is quenched (ca. 93%) after the addition of F\(^-\). The red-shifted emission spectra are attributed to the formation of the negatively charged naphthalimide formed due to the deprotonation of the amine moiety. Thus, new emission band at 580 nm appears. Other anions such as Cl\(^-\), Br\(^-\) and I\(^-\) are not able to deprotonate the amide group.

Another polymeric sensor, which was synthesized by RAFT polymerization, contains the thiourea recognition moiety for anion recognition (Figure 10a).\(^101\) Different anions (AcO\(^-\), H\(_3\)PO\(_4\), F\(^-\) and other halides) were tested as analytes for free receptor 20 and its polymeric derivative P20-F in DMSO solution. The yellow-to-orange colour change was observed upon addition of AcO\(^-\), H\(_3\)PO\(_4\) and F\(^-\) anions to P20-F. In this work, it was observed that not only fluoride, but also other strongly basic anions could deprotonate the NH-group of naphthalimide. At high concentrations orange-to-purple colour change was observed only for F\(^-\) because of the further deprotonation of the thiourea NH-group.

Another interesting example of the probe with dual band response upon fluoride addition is polypolyphenylacetylene containing naphthalimide 21 (Figure 10b).\(^102\) Optical and sensing properties of monomer 21 and polymer P21-F were studied in acetonitrile. Monomer 21 shows typical naphthalimide absorption peak at 360 nm, while P21-F absorbs in a wider range (310–400 nm) due to the \(\pi-\pi^*\) transition of the polypolyphenylacetylene conjugated backbone. However, the fluorescent quantum yield of P21-F was lower than that of free monomer 21. The latter fact was explained by quantitative occurrence of singlet–singlet energy transfer from the polypolyphenylacetylene backbone to the naphthalimide unit in P21-F. Therefore, the fluorescence of the naphthalimide moiety in P21-F was quenched by the intramolecular energy transfer. The mechanism of fluoride sensing was similar to the one discussed in the previous example.

In polymer material P22-St, the authors introduced a thiourea functional group as a hydrogen bond donor group. Monomer 22 was polymerized by free radical copolymerization with styrene (Figure 10a).\(^103\) Addition of fluoride ions into DMF solutions of 22 and P22-St resulted in a colour change from green-yellow to purple. The effects of different halide ions on the fluorescent intensity of a P22-St, as a thin film (35 µm thick), in aqueous medium was also investigated. The rigid and hydrophobic nature of the polymer hinders the diffusion of halide ions to the receptor moiety. Thus, the response time of the polymer was several minutes. Addition of F\(^-\) led to a fluorescent quenching (25%), while in case of Cl\(^-\), Br\(^-\) and I\(^-\) ions the effect on the fluorescence intensity was negligible.

A similar approach for fluoride detection was utilized by Ghosh and co-workers. Receptor 23 was attached to the Merrifield resin in order to produce a sensing material P23-R. The sensor properties were studied in solution, gel and solid states (Figure 11). The gel produced from the polymer changed the colour in DMSO–H\(_2\)O (8:1, v/v) mixture in the presence of fluoride or acetate anions from greenish yellow to deep blue. This colour change indicated a deprotonation process. The treatment of the beads with Ca\(^{2+}\) ions led to regeneration of the starting colour. This cycle can be turned up to three times for F\(^-\), and then the beads lose their efficiency. The change in colour of the beads through treatment with F\(^-\) and Ca\(^{2+}\) was observed due to the deprotonation and reprotonation phenomena.

A poly(ethylene-butyl acrylate) copolymer (EBA) was modified and functionalized by naphthalimide derivative yielding pH sensor P24-E (Figure 12a).\(^104\) At low pH values the aromatic amine of the naphthalimide-derivative was protonated and a decrease in absorbance and the corresponding quenching of fluorescence was observed. The pK\(_a\) values of the free naphthalimide derivative and the polymer, measured in water:ethanol (4:1, v/v) mixture, were different: ~0.88 for P24-E and 1.41 for 24. The sensor P24-E was used to evaluate the molarity of HCl solutions in the range of 1–12 µM.

In order to detect HCl in vapor, the authors suggested improving the hydrophilic properties of the film surface by oxygen plasma treatment. The plasma treatment decreased the response time of the P24-E for HCl from 80 min to 30 min. The vapor penetration into the surface of modified materials was thus much better as the original one.

A fluorescent polymer nanoprobe P25-P-N was proposed to detect HClO by self-assembling of two fluorophores containing amphiphilic block copolymers obtained by a simple co-precipitation method (Figure 12b).\(^105\) Two amphiphilic block copolymers of P25-P-N were covalently linked with porphyrin fluorophore 25P (HClO responsive unit) and naphthalimide fluorophore 25N (reference unit). P25-P-N displayed a single excitation wavelength (405 nm) and two well-resolved emission peaks at 464 (23N) and 655 nm (25P), enhancing the sensitivity. The polymer micelles P25-P-N with an average particle size about 22 nm were stable at 25°C for more than 30 days. The addition of HClO (PBS buffer, pHe = 5.0) resulted in a decrease of the absorption at 418 nm of P25-P-N, indicating the reaction between the porphyrin fluorophore 25P and HClO, while the absorbance of 25N at 380 nm did not change. The fluorescent quenching of P25-P-N was explained by intramolecular heavy atom effect of chlorinated porphyrin structure. The selectivity
experiments of P25-P-N were carried out in the presence of various analytes commonly existed in biological matrices. However, only ClO\(^-\) demonstrated remarkable changes band ratio at 464 and 655 nm with determined LOD 1.99 \(\times\) 10\(^{-6}\) m and response time of 7 min. Due to the good cell-membrane permeability P25-P-N nanoparticles were used to detect exogenous and endogenous hypochlorite in lysosomes.

4. Sensors for Small Organic Molecules

There is a number of small molecules (molar mass < 1000 g mol\(^{-1}\)), which are involved in the metabolism of living organisms and represent targets for the detection. For instance, cancer cells can produce large amounts of hydrogen sulfide and the survival degree of cells depends on the amounts of H\(_2\)S. A series of naphthalimide derivatives with amphiphilic block copolymer poly (2-hydroxyethyl methacrylate)-block-poly(methyl methacrylate) P26-M was recently prepared (Figure 13).\(^{[107]}\) After addition of NaHS to the pH 7.4 PBS solution of the P26-M micelles, the resulting solution demonstrated 20-fold fluorescence enhancement at 528 nm after stirring for 30 min. The micelles showed selectivity for H\(_2\)S over other biological thiols such as glutathione and cysteine. Around cancer tissues, the P26-M micelles exhibited dual characteristics for monitoring H\(_2\)S and H\(_2\)S-triggered charge reversal with the reduction of the azido group. The surface charge of P26-M micelles reversed from negative to positive after interaction with H\(_2\)S. The cellular uptake of doxorubicin-loaded P26-M micelles was increased after interaction with H\(_2\)S due to the electrostatic attraction. This interaction resulted in an increase of the doxorubicin (DOX) release. The P26-M micelles were biocompatible to HeLa cells, while DOX-loaded micelles demonstrated enhanced cytotoxicity in HeLa cells in the presence of H\(_2\)S. Moreover, in vivo experiments with P26-M micelles allowed the authors to image tumours with fluorescence spectroscopy.

Formaldehyde is a well-known carcinogen, which is widely found in aquatic food because of illicit addition or improper storage. In order to detect formaldehyde a robust hydrophilic hydrazino-naphthalimide functionalized chitosan (HN-Chitosan)-based polymeric probe P27-Ch was designed (Figure 14).\(^{[108]}\) The polymer was synthesized by a two-step procedure: 4-bromo-1,8-naphthalic anhydride reacted with the chitosan in DMSO to produce
4-bromo-naphthalimide-functionalized chitosan, which then reacted with excess hydrazine hydrate in ethanol to give the target P27-Ch polymer. Four polymers with different content of naphthalimide moiety were successfully prepared. Polymers with wt = 2% (P27-Ch1), 5% (P27-Ch2), and 10% (P27-Ch3) of the naphthalimide derivative were readily soluble in hydrochloric acid solutions, while the polymer with wt = 33% (P27-Ch4) was only slightly soluble. The latter material was studied to detect formaldehyde. Upon addition of a small amount of HCHO fluorescent intensity was considerably increased. The sensing mechanism is based on the reaction of formaldehyde with the hydrazine group, which leads to the hindering of the PET process. Interestingly, the polymer reacts significantly faster (1 min) as compared to the monomeric compound, which took around 30 min. The authors supposed that the reason for such a fast response is the cooperative effect, which comes from the adjacent hydroxyl groups of the sugar along the polymer chain. The logarithmic linear detection range of P27-Ch3 covers nearly three orders of concentration: from $10^{-6}$ to $10^{-4}$ m, FE = 10, with LOD $5 \times 10^{-8}$ m. The sensing selectivity of P27-Ch3 polymer probe was proven in food and water samples. Analysis of these results leads to a conclusion that the selection of the optimal mass content of the receptor molecule in the polymer, along with the possible cooperative effects are necessary to achieve good sensing properties.

One of the most suitable candidates for the selective determination of organic molecules are MIPs (molecularly imprinted polymers). MIPs are highly cross-linked polymers, which are synthesized in the presence of a target guest molecule working as a template. After removal of this template molecule, a threedimensional recognition “pocket” with complementary to guest size, shape, and non-covalent interactions is formed. MIPs can provide strong affinity and selectivity for a template molecule, mimicking natural antibodies[109].

Two fluorescent cholic acid-imprinted polymers, P28-D and P28-P, were synthesized using 4-dimethylamino-nallyl-naphthalimide 28D and 4-piperazinyl-N-allyl-naphthalimide 28P as the fluorescent functional monomers (Figure 15a,c,d).[110]

Fluorescence of P28-D and 28D in CH$_2$Cl$_2$ decreased with increasing concentrations of cholic acid 0–50 $\times 10^{-6}$ m. Fluorescent quenching was stronger for polymer P28-D than that for the monomer. However, an increase in fluorescence was detected for sensors P28-P and 28P with FE = 2.8 and 1.2 for the polymer and for the receptor, respectively. These changes were explained by the protonation, which in turn suppresses the competitive PET process. The selectivity of both polymers was good against such compounds as cholesterol, testosterone, bisphenol A (BPA) and 2,4-dichlorophenoxyacetic acid (2,4-D)). The imprinted polymers P28-D and P28-P have been successfully applied to the determination of cholic acid in human serums.
A disposable evanescent wave fibre optic sensor was designed by coating a molecularly imprinted polymer (MIP) containing a fluorescent signalling group on a 4-cm long polystyrene optical waveguide. This naphthalimide-based fluorescent MIP showed fluorescence enhancement upon binding the carboxyl-containing molecules. Both monomer 29 and polymer P29-M demonstrated fluorescent enhancement in the presence of carboxyl-containing molecules (in a methanol/water mixture) (Figure 15a,c,e). Blocking the PET process upon binding was suggested as a reason for fluorescence enhancement. The piperazine nitrogen in 29 interacts with the carboxylic group of the analyte by through ionic interactions, which hinders the PET process. The fluorescent enhancement in case of P29-MD was higher than that found for the monomeric one.

Binding studies showed that herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is bound to two different sites with affinities $K_a = 8.5 \times 10^6$ m$^{-1}$ and $K_a = 510$ m$^{-1}$, respectively. The binding of 2,4-D was compared with that of structurally related compounds, 2,4-Dmethylene(2,4-D-OMe), and phenoxyacetic acid (POAc), together with two non-related compounds N-carboen-zyloxy-L-phenylalanine (Z-L-Phe) and $\beta$-D-glucuronic acid (GlcA). The fluorescent intensity of P29-MD studied in the presence of each analyte and resulted in little fluorescence enhancement (<20%), while for 2,4-D it was near 45%. The MIP-coated fibre optic device exhibited even lower limit of quantification (LOQ) of 1 nm, and demonstrated FE = 1.65 with 2,4-D. Next, citrinin MIP particles P29-MC (size 15–500 nm) were synthesized by precipitation polymerization, which showed fluorescence enhancement for even very low concentrations of citrinin. The particles were then coated on the polystyrene waveguide and tested with solutions of citrinin, 2,4-D and FB1 (mycotoxin fumonisin B1) in methanol. The authors observed even in this case a good selectivity for citrinin. It was concluded that herbicide 2,4-D and the mycotoxin citrinin could be selectively determined in solution. The authors believe that their portable device will be useful for cheap and rapid on-site monitoring of environmental, food and biomedical analytes.

A fluorescent MIP P30-M was developed based on naphthalimide 30 as a fluorescent functional monomer (Figure 15a,b) for the detection of caffeine.$^{112}$ Addition of caffeine caused the formation of the hydrogen bonding complex as depicted in Figure 15b and the reduction potential of the receptor was increased, facilitating PET. Thus, fluorescence quenching was observed. Caffeine absorption by MIP P30-M and non-imprinted P30-N was also investigated by HPLC and UV-Vis spectroscopy. The amounts of caffeine adsorbed by P30-M was 1.9 times higher than for P30-N. To test the selectivity of P30-M and P30-N, the caffeine analogues theophiline and theobromine were investigated. Due to the non-specific surface of P30-N only a small decrease in fluorescent intensity
was observed upon addition of caffeine and its analogues. In case of P30-M, only caffeine caused considerable fluorescent quenching. The relative intensity was linear against caffeine concentration from $1.94 \times 10^{-6}$ to $1.94 \times 10^{-4}$ g ml$^{-1}$ with a LOD $1.22 \times 10^{-6}$ g ml$^{-1}$. The P30-M sensor was successfully applied to the determination of caffeine in real samples of tea. Thus, it has been shown that specific binding sites of MIPs provide high selectivity for binding and also have high affinity for a given analyte.

5. Cell Imaging

Amphiphilic polymer nanoparticles P31-N were prepared by grafting a naphthalimide fluorophore onto poly(acrylic acid) for fluorescence imaging of living cells and C. elegans (Figure 16). Two-photon excitation was used, which is more attractive due to the longer wavelength excitation (690–1000 nm). An amphiphilic polymer synthesized by the conjugation of poly(acrylic acid) and naphthalimide derivative 31 and self-assembled during the dialysis process. The experiments in vitro and in vivo showed that P31-N has sufficient biocompatibility and maintained fluorescent properties. P31-N could enter living cells and was primarily located in the cytoplasm. E. coli was used as food source of Nematode worms C. elegans. During the feeding process C. elegans, P31-N were ingested and were recognized and taken up by the active transport system of the intestinal cells. In addition, modifiable carboxyl groups on the P31-N surface could act as a platform to build multifunctional probes for potential applications in biosensing and assay labelling.

Another example of the polymer containing nanoparticles is based on P32-N. This material was used for cell imaging and for therapeutic delivery of prodrugs. Nanoparticles prepared by growing short polymer chains from an aggregation induced emission (AIE) naphthalimide derivative
with the help of nitroxide-mediated polymerization, followed by co-nanoprecipitation of the resulting conjugates with similarly constructed anticancer polymer prodrugs (Figure 16e). The AIE properties of P32-N and monomer naphthalimide derivative 32 were investigated in THF/H2O mixtures. As expected for AIE naphthalimide derivatives, the fluorescence intensity of the polymers increased with increasing water content in the mixture. However, the polymer has slightly lower FE factor. This fact was explained by the partially restricted intramolecular rotations naphthalimide derivative 32 in polymer P32-N. The imaging studies in living cells show accurate intracellular visualization in different cell lines.
and good biocompatibility of the polymer nanoparticles. AIE-active polymer prodrug nanoparticles were prepared by co-nanoprecipitation of P32-N (10.8 wt%) with cladribine-diglycolatepolyisoprene (CdA-digly-PI), a well-defined anticancer polymer. Prodrug nanoparticles demonstrated the possibility for fluorescent labelling with an AIE active naphthalimide derivative for imaging and potential application in diagnosis and therapeutic delivery of prodrugs.

The over-expressed nitroreductase (NTR) is a common biomarker of tumor hypoxia. Tang and coworkers designed ratiometric fluorescent biosensor P33-P for the sensing of NTR and diagnosis of hypoxia in tumor cells (Figure 17).\textsuperscript{[112]} 1,8-Naphthimide was covalently attached to the side chain of polyfluorene-co-phenylene (PFP) and modified with p-nitrobenzene, that acted as both a quencher and a recognition unit. Because of the FRET from main chain to naphthalimide dye and the PET between naphthalimide-derivative and nitrobenzene group the optical background signal of the probe is low. In the presence of NTR the nitro group is reduced to an amino group, leading to PET being blocked and the ratio

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Figure 15. a) Structures of receptors 28D, 28P, 29, and 30. b) Proposed hydrogen bonding interactions between caffeine and P30-M. c) Structures of cholic acid, 2,4-dichlorophenoxyacetic acid (2,4-D) and Citrinin. d) Schematic illustration of the molecular imprinted polymer P28-D. e) Schematic illustration of the polystyrene evanescent wave fibre optic wave guide coated with fluorescent MIP (P29-MD and P29-MC) particles.
$I_{526}/I_{417}$ (33:PFP) greatly increases (> 54-fold), with LOD 19.7 ng mL$^{-1}$. Moreover, the fluorescence of the PFP backbone can be used as an internal reference while remains at the same level. It is important that ratiometric probe P33-P exhibits high selectivity and low cytotoxicity, and can achieve intracellular detection of NTR and hypoxia diagnosis in tumor cells.
Figure 17. Schematic structure of P33-P. Images of co-localization of P33-P and Lyso-Tracker Red in A549 cells. The fluorescence imaging of PFP channel, NA channel and Lyso-Tracker Red channel were carried out at 410–460 nm (λex = 405 nm), 500–550 nm (λex = 405 nm), and 575–620 nm (λex = 559 nm), respectively. Scale bar is 40 µm. The detection mechanism of P33-P (PFP-NA) toward NTR is also shown. Reproduced with permission.[115] Copyright 2016, Elsevier.

6. Conclusions

As can be inferred from the analysed literature data, two main approaches are implemented to design functional sensing polymers. In the first approach, the fluorophore is covalently attached to the polymer matrix, usually at the stage of radical polymerization. This method was used to obtain sensors based on various esters of polymethacrylic acid, N,N-dimethylacrylamide and the corresponding allyl or methacrylic derivatives of 1,8-naphthalimide. Although, this method benefits from the
Table 2. Summary of fluorescence sensors for different species.

| Target species | Polymer name | Monomer structure | Polymerization technique/Reagents | Solvent | Used as | λ<sub>a</sub> [nm] | λ<sub>f</sub> [nm] | Sensitivity, LOD<sup>a</sup> [μM] |
|----------------|--------------|-------------------|----------------------------------|---------|---------|----------------|----------------|-------------------------------|
| Fe<sup>3+</sup> | P1-MMA       | ![Monomer structure P1-MMA](image) | FRP<sup>b</sup>/MMA             | DMF     | Solution| 428            | 495, 515        | 10<sup>−4</sup>                 |
| Fe<sup>3+</sup> | P1-St        | ![Monomer structure P1-St](image) | FRP/Styrene                      | Acetate buffer, pH = 5 | Film   | 398            | 497            | 10<sup>−4</sup>                 |
| Fe<sup>3+</sup> | P2-MMA       | ![Monomer structure P2-MMA](image) | FRP/MMA (DBP)                    | Acetate buffer, pH = 5 | Film   | 416            | 476            | 10<sup>−4</sup>                 |
| Fe<sup>3+</sup> | P2-St        | ![Monomer structure P2-St](image) | FRP/Styrene (DBP)                | Acetate buffer, pH = 5 | Film   | 426            | 478            | 10<sup>−4</sup>                 |
| Fe<sup>3+</sup> | P3-St        | ![Monomer structure P3-St](image) | FRP/Styrene                      | H<sub>2</sub>O | Film   | 375            | 421            | 2 × 10<sup>−4</sup> −5 × 10<sup>−5</sup> |
| Fe<sup>3+</sup> | P4-St        | ![Monomer structure P4-St](image) | FRP/Styrene                      | H<sub>2</sub>O | Film   | 373            | 428            | 2 × 10<sup>−4</sup> −5 × 10<sup>−5</sup> |
| Fe<sup>3+</sup> | P5-G         | ![Monomer structure P5-G](image) | Photo-copolymeriz./HEMA (init. benzoin ethyl ether, benzophenone inh. triethanolamine) | 0.05M Tris/HCl, pH = 6 | Quartz glass plate | 408            | 521            | 4.5 × 10<sup>−4</sup>         |
| Fe<sup>3+</sup> | P6-R         | ![Monomer structure P6-R](image) | Attach/ DCC, HOBt, TentaGels, MB-NH₂ | 1:1 (v/v) MeOH/H<sub>2</sub>O, pH 4 | Polym. bead (monm) | 379            | 523 (monm)       | 2 × 10<sup>−3</sup>          |

<sup>a</sup> LOD: Limit of Detection.
| Target species | Polymer name | Monomer structure | Polymerization technique/Reagents | Solvent Used as | λ$_{A}$ [nm] | λ$_{F}$ [nm] | Sensitivity, LOD$_{a}$ [m] | Ref. |
|----------------|--------------|-------------------|----------------------------------|-----------------|-------------|-------------|----------------|-----|
| Fe$^{3+}$ P7-R | Attach/ DCC, HOBt, TentaGels, MB-NH$_2$ | 1:1 (v/v) MeOH/H$_2$O, pH 4 | Polym. bead | 382 | 520 | $5 \times 10^{-5}$ | [75] |
| Fe$^{3+}$ P8_Rh | RAFT/ DDMAT, (AIBN) Britton-Robinson buffers | Solution | 400 | I$_{555}$/I$_{520}$ | $5 \times 10^{-5}$ | [76] |
| Fe$^{3+}$ P8-T | Attach/Cul, Piperidine, poly[3-(6-bromohexyl) thiophene] | 10:1 (v/v) CHCl$_3$/MeOH | Solution | 401 | 535 | $1 \times 10^{-8}$ | [77] |
| Fe$^{3+}$/Zn$^{2+}$ P9-H | Attach/CuSO$_4$ (+)Na L-ascorbate, Hybrane P1000 hyperbranched polymer | CH$_3$CN | Solution | 433 | 526 | $10^{-6}$ | [78] |
| Cu$^{2+}$ P10-G | Photo-copolymeriz./HEMA (init. benzoil ethyl ether, benzophenone inh. triethanolamine) | Tris–HCl, pH 7.24 Quartz glass plate | 453 | 514 | $10^{-5}$ | [81] |
| Cu$^{2+}$ P11-NP | Therm. polymerization/(AIBN) MMA | Water Nano-particle dispersion | 420 | 505 | $10^{-5}$ | [82] |
| Target  
| species | Polymer  
| name | Monomer  
| structure | Polymerization  
| technique/  
| Reagents | Solvent | Used as | $\lambda_a$ [nm] | $\lambda_f$ [nm] | Sensitivity, LOD$^{10}$ [mL] | Ref. |
| Hg$^{2+}$ | P12-G | Photo-copolymeriz./ HEMA (init. benzoin ethyl ether, benzophenone inh. triethanolamine) | Tris–HCl, $5 \times 10^{-3}$ m, pH 7.01 | Quartz glass plate | 420 | 520 | $2 \times 10^{-6}$ | [83] |
| Hg$^{2+}$ | P13-M | Attach/ | 4:1 (v/v) H$_2$O/ EtOH, pH 7.0 | Membrane | 407 | 512 | $2 \times 10^{-6}$ | 1 | [84] |
| K$^+$ | P14-G | Therm. polymerization/ (AIBN) METAC, MESA, AM, HEMA | $10 \times 10^{-3}$ m HEPES buffer (pH 7.4) | Quartz glass plate | 450 | 525 | — | [86] |
| Ba$^{2+}$/Ca$^{2+}$ | P15-HG | FRP/ DMA, MBA, (init: APS, TMEDA) | CH$_3$CN Solution | 424 | 522 | — | [88] |
| Ca$^{2+}$ | P16-C | Attach/ N-hydroxysuccinimide, activated cellulose | 0.1 m HEPES, pH 7.4 | Film LED end at 480 nm cut-off | 520-emiss. | $2 \times 10^{-6}$ | [90] |
| Target species | Polymer name | Monomer structure | Polymerization technique/Reagents | Solvent | Used as | $\lambda_{s}$ [nm] | $\lambda_{f}$ [nm] | Sensitivity, LOD$^{a}$ [m] | Ref. |
|---------------|-------------|-------------------|-----------------------------------|---------|---------|-------------------|-------------------|------------------------|-----|
| Na$^+$        | P17-C       | ![Monomer structure](image) | Attach/                           | 0.1 M HEPES, pH 7.4 | Film    | 470              | 560              | $10^{-3}$               | [93]|
| F$^-$         | P18-F       | ![Monomer structure](image) | RAFT/CDB, (AIBN)                | CH$_2$Cl$_2$-DMSO (9:1 v/v) | Films on the quartz plates | 398 (pure), 500 (compl. F$^-$) | 484               | $10^{-3}$               | [100]|
| F$^-$         | P19-F       | ![Monomer structure](image) | RAFT/CDB, (AIBN)                | DMSO     | Films on the quartz plates | 408 (ca. 440 compl. F$^-$) | N.M.              | $10^{-3}$               | [101]|
| F$^-$         | P20-F       | ![Monomer structure](image) | Cat. Polym/[(Rh[nbd]Cl)$_2$]     | CH$_3$CN | Solution | 360 (pure), 490 (compl. F$^-$) | 460 (pure), 580 (compl. F$^-$) | $2 \times 10^{-4}$ | [102]|
| F$^-$         | P21-St      | ![Monomer structure](image) | FRP/ Styrene (DBP)              | Water    | Film    | 376              | 505              | $10^{-3}$               | [103]|

Table 2. Continued.
| Target species | Polymer name | Monomer structure | Polymerization technique/Reagents | Solvent | Used as | $\lambda_a$ [nm] | $\lambda_r$ [nm] | Sensitivity, LOD$^{a}$ [m] | Ref. |
|----------------|-------------|-------------------|----------------------------------|---------|---------|-----------------|-----------------|----------------------------|------|
| $F^-$, $AcO^-$ | P22-R       | ![Monomer structure](image) | Attach / Merrifield resin | CH$_3$CN | Polym. bead | 435 | 507 | monom | $3 \times 10^{-7}$ | [104] |
| HCl            | P23-E       | ![Monomer structure](image) | Attach / EBA-COCI, triethylamine | pH 7 to $-1.1$ (12M HCl, 37%) | Film | 327, 348 (acid), 370 (base), 480 (acid), 488 (base) | – | – | [105] |
| HClO           | P24-P-N     | ![Monomer structure](image) | Polymer micelle | PBS buffer, pH 5.0 | Micelle | 380 (napht), 418 (porf), 464 (napht), 655 (porf) | $10^{-4}$ | 10$^{-4}$ | [106] |
| H$_2$S         | P25-M       | ![Monomer structure](image) | Polymer micelle dialysis method poly(2-hydroxyethyl methacrylate)-block-poly(methylmethacrylate) | PBS buffer, pH 7.4 | Micelle | 438 | 528 | $10^{-5}$ | [107] |
| HCHO (formaldehyde) | P26-Ch   | ![Monomer structure](image) | Attach/Chitosan | 0.1M HCl, water | Solution | 440 | 555 | $10^{-6}$ | [108] |
| Cholic acid    | P27-D       | ![Monomer structure](image) | MIP/ cholic acid, acrylamide, EGDMA, (AIBN) | CH$_2$Cl$_2$ | Solution | 415 | 504 | $10^{-6}$ | [110] |
| Cholic acid    | P27-P       | ![Monomer structure](image) | MIP/ cholic acid, acrylamide, EGDMA, AIBN | CH$_2$Cl$_2$ | Solution | 427 | 510 | $10^{-8}$ | [110] |
| Target species | Polymer name | Monomer structure | Polymerization technique/Reagents | Solvent | Used as | $\lambda_a$ [nm] | $\lambda_F$ [nm] | Sensitivity, LOD$^a$ [m] | Ref. |
|----------------|-------------|------------------|----------------------------------|---------|---------|-----------------|-----------------|------------------|------|
| 2,4-dichlorophenoxyacetic acid | P28-MD | | MIP/4-dichlorophenoxyacetic acid, 4-VPY, EGDMA, ABDV | MeOH/H$_2$O, (4/1) | Particles, Fibers | 390 | 515 | $10^{-4}$ | [111] |
| | | | | | | | 410 | 515 | $10^{-4}$ | |
| Citrinin | P28-MC | | MIP/citrinin, 4-VPY, EGDMA, ABDV | MeOH | Particles, Fibers | 390 | 515 | $10^{-4}$ | [111] |
| | | | | | | | 410 | 515 | $10^{-5}$ | |
| Caffeine | P29-M | | MIP/Caffeine, AIBN, MAA, EGMA | DMF, buff. solution, pH 7 | Solut. and solid form | 392 | 517 | $10^{-6}$ | [112] |
| Cell imaging | P30-N | | Attach/ poly(acrylic acid) | PBS buffer | Nano-partic. | 444 | 540 | – | [113] |
| Cell imaging | P31-N | | Attach + nitrooxide-mediated polymerization, alkoxyamine bearing monomer, isoprene | H$_2$O/THF mixtures and PBS buffer | Nano-partic. | 420 | 490 | – | [114] |
Conjugated nitrogen atom in the 4th position of the naphthalimide ring, sensors have been synthesized. All considered sensors contain a fluorophore based on relatively simple structures of naphthalimide derivatives. However, to date, polymers of the fluorophore are carried out using click reactions with aminocellulose fibers. In this case, the covalent incorporation of the free receptor makes it possible to determine the analyte not only in organic, but also in aqueous media. Several challenges are still to be solved in near future. For example, the loss of the optical response (for example, due to the cooperative effect) often limits the sensor sensitivity, improvement of selectivity, and some properties of a starting molecular probe is not a trivial task. Nevertheless, work in this direction is actively ongoing by exploring new ways of construction of polymeric sensors with retained properties of the free receptor.

Despite the large number of naphthalimide-based sensors, construction of polymeric sensors with retained properties of the monomer remains a challenging task. Nevertheless, work in this direction is actively ongoing by exploring new ways of polymer synthesis and functionalization.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

fiber-optic sensor, fluorescence, fluorescent polymer, molecular probe, naphthalimide

Table 2. Continued.

| Target species | Polymer name | Monomer structure | Polymerization technique/Reagents | Solvent Used as | λλ [nm] | λF [nm] | Sensitivity, Ref. |
|----------------|--------------|-------------------|----------------------------------|-----------------|-------|-------|-----------------|
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