Sensitive Hg\textsuperscript{2+} Sensing via Quenching the Fluorescence of the Complex between Polythymine and 5,10,15,20-tetrakis(N-methyl-4-pyridyl) Porphyrin (TMPyP)

Daohong Wu, Yaliang Huang, Shengqiang Hu, Xinyao Yi * and Jianxiu Wang

College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China; wudaohong@csu.edu.cn (D.W.); 182301010@csu.edu.cn (Y.H.); zn_sqhu@csu.edu.cn (S.H.); jxiuwang@csu.edu.cn (J.W.)

* Correspondence: yixinyao@csu.edu.cn; Tel.: +86-188-7402-6411

Received: 26 October 2018; Accepted: 14 November 2018; Published: 16 November 2018

Abstract: The interaction between polythymine (dTn) and 5,10,15,20-tetrakis(N-methyl-4-pyridyl) porphyrin (TMPyP) was systematically studied using various techniques. dTn remarkably enhanced the fluorescence intensity of TMPyP as compared to other oligonucleotides. The enhanced fluorescence intensity and the shift of the emission peaks were ascribed to the formation of a π-π complex between TMPyP and dTn. And the quenching of the dTn-enhanced fluorescence by Hg\textsuperscript{2+} through a synergistic effect occurs due to the heavy atom effect. The binding of Hg\textsuperscript{2+} to TMPyP plays an important role in the Hg-TMPyP-dT\textsubscript{30} ternary complex formation. A TMPyP-dT\textsubscript{30}-based Hg\textsuperscript{2+} sensor was developed with a dynamic range of Hg\textsuperscript{2+} from 5 nM to 100 nM. The detection limit of 1.3 nM was low enough for Hg\textsuperscript{2+} determination. The sensor also exhibited good selectivity against other metal ions. Experiments for tap water and river water demonstrated that the detection method was applicable for Hg\textsuperscript{2+} determination in real samples. The Hg\textsuperscript{2+} sensor based on oligonucleotide dT\textsubscript{30}-enhanced TMPyP fluorescence was fast and low-cost, presenting a promising platform for practical Hg\textsuperscript{2+} determination.

Keywords: interaction; polythymine; TMPyP; Hg\textsuperscript{2+}; synergistic effect; ternary complex

1. Introduction

As one of the most important conjugated organic molecules, porphyrins play a crucial role in the metabolism of living organisms [1]. For example, porphyrins are responsible for the production of singlet oxygen, which could damage DNA in tumor cells [2]. The interaction between porphyrins or their derivatives and nucleic acids has been extensively investigated [3–5], in which three binding types including intercalation, external or groove binding, and outside stacking were involved [6].

5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphyrin (TMPyP) is a water-soluble cationic porphyrin, which contains a porphyrin core and N-methylpyridinium side chains [7]. Due to their planarity and hydrophobicity, the porphyrin rings can intercalate into the base pairs of DNA and in the same time, the positively charged side chains can electrostatically interact with the negatively charged nucleic acids [8]. For example, evidenced by nuclear magnetic resonance spectroscopy, TMPyP can intercalate into GC-rich regions of duplex DNA [9,10]. However, the binding of TMPyP to the major groove of the AT-rich regions has also been proposed [11]. TMPyP has been reported to interact with triplex DNA, in which the third strand could inhibit the assembly of TMPyP with duplex DNA [12]. TMPyP can also bind to the G-quadruplex structure through external stacking rather than intercalation between...
the G-tetrads [13–15]. As probably the most flexible form, the single stranded DNA enables unrivaled access to individual bases [6], which facilitates the interaction with TMPyP.

Mercuric ion (Hg$^{2+}$) possesses serious immunotoxic, genotoxic, and neurotoxic effects, causing damage to the central nervous system, endocrine system and other organs such as the kidney, liver, heart, and lung [16]. Hg$^{2+}$ usually serves as a fluorescent quencher through enhancement of spin-orbit coupling [17]. A colorimetric method for Hg$^{2+}$ determination through platinum nanoparticles with limit of detection 8.5 pM and a linear range from 0.01 to 4 nM has been reported by Aragay et al. [18]. MoS$_2$ nanosheet/DNA/carbon dot-based fluorescence method has been reported to detect Hg$^{2+}$ [19]. However, most of the methods were based on synthesized nanometer materials such as nanoparticle, quantum dots, nanosheet, which were hard to be modified. Herein, the interaction between TMPyP and polyadenine (dA$_n$), polycytosine (dC$_n$), polyguanine (dG$_n$) and polythymine (dT$_n$) were investigated. dT$_n$ was found to substantially enhance the fluorescence intensity of TMPyP due to the formation of a TMPyP-dT$_n$ complex. The incorporation of Hg$^{2+}$ significantly quenched the fluorescence of the complex and a fluorescent sensor for Hg$^{2+}$ was developed. The sensing strategy is shown in Figure 1.

![Figure 1. Schematic of Hg$^{2+}$ assay by quenching the fluorescence of TMPyP-dT$_{30}$.](image)

2. Materials and Methods

2.1. Reagents and Chemicals

5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphyrin (TMPyP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The oligonucleotides (ODNs), such as polyadenine dA$_{13}$, polycytosine dC$_{13}$, polyguanine dG$_6$TG$_6$ and polythymine dT$_5$, dT$_{10}$, dT$_{15}$, dT$_{15}$, dT$_{20}$, dT$_{30}$, dT$_{40}$, dT$_{50}$ were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). Hg(NO$_3$)$_2$, Cd(NO$_3$)$_2$·4H$_2$O, Cr(NO$_3$)$_3$·9H$_2$O, Cu(NO$_3$)$_2$·3H$_2$O, Fe(NO$_3$)$_3$·9H$_2$O, FeSO$_4$·7H$_2$O, Mn(CH$_3$COO)$_2$·4H$_2$O, MgCl$_2$·6H$_2$O, NiCl$_2$·6H$_2$O, Pb(NO$_3$)$_2$, ZnCl$_2$, Tris-HCl, CH$_3$COOH, NaCl and NaOH were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the ODNs were prepared with Tris-HCl buffer (10 mM Tris, 100 mM NaCl, pH 7.4). All reagents were of analytical grade and used as received. Ultrapure water was obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore).

2.2. Apparatus

UV-vis spectra and circular dichroism (CD) spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan) and a Jasco-815 spectropolarimeter (Jasco, Japan), respectively. Fluorescence spectra were collected on a Hitachi F-4600 spectrofluorometer (Hitachi, Japan). Fluorescence lifetime was measured on a compact FluoTime 100 fluorescence lifetime spectrometer (PicoQuant GmbH, Germany).
2.3. Procedure

The TMPyP-ODN complex was formed by mixing 1 µM TMPyP and 2 µM ODN, followed by dilution to 200 µL with Tris-HCl buffer and stirring for 50 min. For the Hg²⁺ assay, Hg²⁺ with various concentrations was added into the solution of TMPyP-dT₃₀ complex under stirring for 1 min. For the fluorescence, measurement with excitation was 422 nm and emission was at 660 nm. The fluorescence lifetimes were determined from the data obtained from a compact FluoTime 100 fluorescence lifetime spectrometer. The data could be analyzed by exponential fits. The solution of Hg²⁺, TMPyP and dT₃₀ were mixed by 1 µM Hg²⁺ and 1 µM TMPyP for Hg-TMPyP; 1 µM TMPyP and 2 µM dT₃₀ for TMPyP-dT₃₀; 1 µM Hg²⁺, 1 µM TMPyP and 2 µM dT₃₀ for Hg-TMPyP-dT₃₀. The solutions were followed by dilution to 200 µL with Tris-HCl buffer and stirring for 120 min. All the lifetime measurements were with an excitation at 422 nm.

3. Results and Discussion

3.1. Interaction between ODNs and TMPyP

The interaction between TMPyP and ODNs was characterized by fluorescence and UV-vis absorption spectroscopy. As shown in Figure 2A, upon excitation at 422 nm, TMPyP exhibited two broad peaks at around 665 nm and 715 nm (curve a). With the addition of dG₆TG₆, the fluorescence of TMPyP decreased due to the electron transfer from guanine to TMPyP (curve b) [20]. However, the incorporation of dA₁₃ (curve c), dC₁₃ (curve d) and dT₁₃ (curve e) increased the fluorescence of TMPyP, and the emission wavelengths were shifted to 660 nm and 720 nm. The enhanced fluorescence intensity and the shift of the emission peaks were ascribed to the formation of a π-π complex between TMPyP and ODNs [20]. The above results were consistent with those in the cases of mononucleotides. Mononucleotides interacted differently with TMPyP, in which adenine, thymine, and cytosine increased the fluorescence intensity of TMPyP, while guanine substantially quenched the fluorescence intensity of TMPyP [20]. The higher fluorescence of TMPyP by dT₁₃ than that by dA₁₃ or dC₁₃ indicated that TMPyP binded to dT₁₃ with stronger affinity [21].

Figure 2. (A) Fluorescence and (B) UV-vis absorption spectra of 1.0 µM TMPyP in the (a) absence and (b) presence of 2.0 µM dG₆TG₆, (c) dA₁₃, (d) dC₁₃ or (e) dT₁₃.

TMPyP displayed a characteristic absorption peak at 422 nm known as the Soret band (curve a in Figure 2B), which originated from the S₀–S₂ transition [22]. The incorporation of dG₆TG₆ (curve b), dA₁₃ (curve c), dC₁₃ (curve d) and dT₁₃ (curve e) decreased the absorption of TMPyP, and the absorbance peak of TMPyP was red-shifted to 426 nm and 431 nm in the case of dC₁₃ (curve d) and dT₁₃ (curve e), respectively. The larger bathochromic shift in curve e suggested the higher binding affinity between TMPyP and dT₁₃.

The length of polythymine also influenced the fluorescence enhancement of TMPyP (Figure 3). The incorporation of different lengths of polythymine resulted in increased fluorescence and began to level off with the length of dT₃₀. Thus, the length of dT₃₀ might have been more favorable for π-π stacking between TMPyP and polythymine.
Sensors 2018, 18, 3998

Figure 3. Dependence of fluorescence intensity on the sequence length of poly T. The fluorescence was excited at 422 nm and measured at 660 nm. The absolute errors deduced from three replicate measurements are shown as the error bars.

3.2. Fluorescence Quenching of TMPyP-dT$_{30}$ Complex by Hg$^{2+}$

In comparison with the fluorescence of TMPyP at 665 nm and 715 nm (curve a in Figure 4A), the fluorescence of the TMPyP-dT$_{30}$ complex at 660 nm and 720 nm (curve b in Figure 4A) was significantly enhanced. With the addition of Hg$^{2+}$, the fluorescence of the TMPyP-dT$_{30}$ complex at 660 nm was greatly quenched and in the same time, a tiny and broad emission peak at 635 nm was attained (curve c). The emission peak at 635 nm was ascribed to that of Hg-TMPyP, as evidenced by the emission of Hg-TMPyP in curve d. Porphyrins have been reported to bind to Hg$^{2+}$ with the formation of metalloporphyrin [23], and the fluorescence of porphyrins could be quenched by Hg$^{2+}$ due to the heavy atom effect [24]. Based on the results above, a new ternary complex of Hg-TMPyP-dT$_{30}$ might have formed [25], providing the possibility for a Hg$^{2+}$ assay.

Figure 4. (A) Fluorescence and (B) UV-vis absorption spectra of (a) 1.0 μM TMPyP, (b) 1.0 μM TMPyP + 2.0 μM dT$_{30}$, (c) 1.0 μM TMPyP + 2.0 μM dT$_{30}$ + 4.0 μM Hg$^{2+}$, (d) 1.0 μM TMPyP + 4.0 μM Hg$^{2+}$. (C) CD spectra of (a) 20 μM dT$_{30}$, (b) 20 μM dT$_{30}$ + 100 μM TMPyP, (c) b + 100 μM Hg$^{2+}$, (d) a + 100 μM Hg$^{2+}$. (D) Fluorescence spectra of (a) 1.0 μM TMPyP + 2.0 μM dC$_{30}$, (b) a + 4.0 μM Hg$^{2+}$. The fluorescence was excited at 422 nm and measured at 660 nm.
To further demonstrate the formation of the ternary complex, UV-vis absorption spectra were investigated (Figure 4B). With the addition of dT₃₀, the Soret band of TMPyP at 422 nm (curve a) was red-shifted to 431 nm (curve b). The electrostatic interaction and π-π stacking facilitated the formation of the TMPyP-dT₃₀ complex [20]. The formation of the ternary complex of Hg-TMPyP-dT₃₀ led to a larger bathochromic shift of the Soret band to 462 nm, and in the same time, a new and small peak appeared at 437 nm (curve c). The new peak originated from the absorption of Hg-TMPyP (curve d) [26].

The conformational change of dT₃₀ induced by TMPyP was studied by CD measurements (Figure 4C). The dT₃₀ exhibited a negative peak at 250 nm and a positive peak at 275 nm (curve a). Upon adding TMPyP to the solution of dT₃₀, both peaks decreased slightly and in the same time, a new positive peak at 430 nm appeared (curve b), which indicated the binding of TMPyP to dT₃₀ through π-π stacking [27,28]. Note that TMPyP did not show any detectable CD signal. With the incorporation of Hg²⁺ to the TMPyP-dT₃₀ complex, the 275 nm-peak disappeared and the negative peak at 250 nm shifted to 260 nm (curve c). Furthermore, the 430 nm-peak disappeared. The results above suggested that the binding of TMPyP to dT₃₀ was interrupted by Hg²⁺. Such an interruption might have been ascribed to the alteration of the planarity of the TMPyP core induced by Hg²⁺ [3]. When adding Hg²⁺ to dT₃₀ solution, the formation of the T–Hg–T complex was characterized by the negative peak at 255 nm and the positive peak at 280 nm (curve d). No positive peak at 280 nm in curve c was shown compared to the T–Hg–T complex, which indicated the different structure of dT₃₀ in T–Hg–T and the Hg-TMPyP-dT₃₀ complex.

The fluorescence lifetime measurement provided deep insight into the ternary complex formation. The lifetime of TMPyP determined from the decay curve was 4.6 ns, consistent with that reported previously [26]. The lifetime was increased to 10.2 ns upon formation of TMPyP-dT₃₀. However, the ternary complex of Hg-TMPyP-dT₃₀ possessed a much shorter lifetime of 2.0 ns. Upon adding Hg²⁺ to the solution of TMPyP, a lifetime of 1.2 ns was observed, indicating the binding of Hg²⁺ to TMPyP [26].

As reported previously, the binding of TMPyP to dT₃₀ possessed high binding affinity (binding constant of 10⁷ M⁻¹), while the coordination of Hg²⁺ to dT₃₀ was a slow dynamic process at low concentrations of Hg²⁺ [29]. The binding of Hg²⁺ to TMPyP plays an important role in the Hg-TMPyP-dT₃₀ ternary complex formation. Upon addition of Hg²⁺ to the solution of TMPyP-dC₃₀, the ternary complex of Hg-TMPyP-dC₃₀ was also formed, as evidenced by the quenching of the fluorescence of TMPyP-dC₃₀ by Hg²⁺ (Figure 4D). Considering the relatively lower affinity between Hg²⁺ and dC₃₀ than that between Hg²⁺ and TMPyP [26,30], the coordination of Hg²⁺ to TMPyP was essential for the formation of the Hg-TMPyP-dC₃₀ complex. The quenching of the fluorescence of TMPyP-dT₃₀ by Hg²⁺ provided the possibility for a Hg²⁺ assay.

3.3. Calibration Curve of The Hg²⁺ Assay

The fluorescence intensity of TMPyP-dT₃₀ at 660 nm gradually decreased with an increased Hg²⁺ concentration (Figure 5A). The dependence of (F₀ − F)/F₀ on the concentration of Hg²⁺ was shown in Figure 5B, where F₀ and F represented the fluorescence intensities in the absence and presence of Hg²⁺, respectively. The inset of Figure 5B depicted the linear portion of the curve with Hg²⁺ concentrations ranging from 5 nM to 100 nM and the linear regression equation was presented as (F₀ − F)/F₀ = 3.22 [Hg²⁺] (µM) + 0.05 (R² = 0.9605). The detection limit was estimated to be 1.3 nM, being much lower than those by the 8-amino BODIPY-based fluorescence assay (49 nM) [31], Raman spectroscopic method (10 nM) [32], colorimetric assay employing plasmonic gold nanoparticles (50 nM [33] and 17.3 nM [34]), colorimetric assay based on Cu₂Se nanoparticles (2.7nM) [35] and the fluorescence polarization assay based on CdTe/CdS quantum dots (8.6 nM) [36]. Such a concentration level was also comparable with those by fluorescent assay based on phosphorothioate RNA modifications (1.7 nM) [37]. The comparison of the proposed sensor with other methods is shown in Table 1. Because the maximum permissible level of Hg²⁺ in drinking water and food, set by the U.S. Environmental
Protection Agency and World Health Organization, is at 2 ppb (10 nM) [38], the sensing protocol serves as a viable alternative for a practical Hg\(^{2+}\) assay.

**Table 1.** A comparison of the proposed sensor with other detection methods.

| Method                      | Tool                                  | Linear Range   | LOD             | Time   | Real Sample                | Ref.        |
|-----------------------------|---------------------------------------|----------------|-----------------|--------|----------------------------|-------------|
| colorimetric                | platinum nanoparticles                 | 0.01–4 nM      | 0.0085 nM       | 10 min | tap water                  | [18]        |
| colorimetric                | gold nanoparticles                     | 25–750 nM      | 50 nM           | 40 min | pond and river water       | [33]        |
| colorimetric                | Cu\(_{2}\)Se nanoparticles              | 0–800 nM       | 2.7 nM          | 10 min | tap, pond and river water  | [35]        |
| colorimetric                | gold nanoparticles and aptamer         | 10–1000 nM     | 17.3 nM         | 20 min | tap, rivers, lakes and ocean water | [34] |
| Raman spectroscopy          | silver nanoparticles                   | 1–1000 µM      | 10 nM           | 30 min | drinking mineral water     | [32]        |
| fluorescence                | Phosphorothioate RNA Modifications     | 0–50 nM        | 1.7 nM          | 20 min | lake water                 | [37]        |
| fluorescence                | MoS\(_2\) nanosheet/DNA/carbon dot     | 0–10 nM        | 1.02 nM         | 15 min | tap and lake water         | [19]        |
| fluorescence                | fluorescent 8-amino BODIPY-based probe| 0.5–5 µM       | 49 nM           | 50 min | SMMC-7721 cells           | [31]        |
| fluorescence polarization   | CdTe/CdS QDs                           | 10–800 nM      | 8.6 nM          | 2.0 h  | lake and spiked lake water| [36]        |
| fluorescence                | polyT-TMPyP                            | 5–100 nM       | 1.3 nM          | 60 s   | Tap and river water        | this work   |

**3.4. Selectivity of the Assay**

The selectivity of the method was evaluated by the addition of various metal ions of 4.0 µM (Figure 6). Hg\(^{2+}\) quenched about 90% of the fluorescence intensity of the TMPyP-dT\(_{30}\) complex at 660 nm. However, other metal ions, such as Cu\(^{2+}\), Fe\(^{3+}\), Fe\(^{2+}\), Cd\(^{2+}\), Cr\(^{3+}\), Mg\(^{2+}\), Mn\(^{2+}\) and Ni\(^{2+}\) exerted negligible influence on the fluorescence intensity of TMPyP-dT\(_{30}\). Pb\(^{2+}\) caused 30% quenching of the fluorescence intensity due to the formation of the unstable Pb\(^{2+}\)-TMPyP-dT\(_{30}\) [39]. The proposed fluorescence method possessed good selectivity toward Hg\(^{2+}\) determination.

**3.5. Practical Samples for the Hg\(^{2+}\) Assay**

The practical application of this method for Hg\(^{2+}\) determination was evaluated by the standard addition method through adding different concentration of Hg\(^{2+}\) in tap water and river water. As shown in Table 2, the recoveries were between 96–105%. The recovery suggested that the method was
largely free from the matrix effect of the complex real water samples and could be served for Hg$^{2+}$ determination in tap water and river water.

![Figure 6. Selectivity of the fluorescence assay of Hg$^{2+}$.](image)

The fluorescence quenching capability of Hg$^{2+}$ on the TMPyP-dT$_{30}$ has been proposed, enabling a sensitive and selective Hg$^{2+}$ assay. The formation of the Hg-TMPyP-dT$_{30}$ ternary complex was characterized by various techniques. The mechanism inherent in the fluorescence quenching of TMPyP-dT$_{30}$ by Hg$^{2+}$ involved a synergistic effect. The detection limit of the proposed method was obtained as 1.3 nM. Experiments for tap water and river water demonstrated that the detection method was applicable for Hg$^{2+}$ determination in real samples. The Hg$^{2+}$ sensor based on oligonucleotide dT$_{30}$ enhanced TMPyP fluorescence was fast and low-cost, presenting a promising platform for practical Hg$^{2+}$ determination. In addition, this is the first time dT$_{30}$ enhanced TMPyP fluorescence was fast and low-cost, presenting a promising platform for practical Hg$^{2+}$ determination. In addition, this is the first time dT$_{30}$ enhanced TMPyP fluorescence was fast and low-cost, presenting a promising platform for practical Hg$^{2+}$ determination.

![Table 2. Determination of Hg$^{2+}$ in water samples with the proposed method.](table)

| Sample       | Added (nM) | Measured (nM) | Recovery (%) |
|--------------|------------|---------------|--------------|
| tap water    | 2.0        | 2.1 ± 0.17    | 105 ± 0.085  |
|              | 5.0        | 4.9 ± 0.32    | 98 ± 0.064   |
|              | 10.0       | 9.6 ± 0.62    | 96 ± 0.062   |
| river water  | 2.0        | 2.1 ± 0.11    | 105 ± 0.055  |
|              | 5.0        | 5.1 ± 0.43    | 102 ± 0.086  |
|              | 10.0       | 9.7 ± 0.65    | 97 ± 0.065   |

*Mean values and standard deviations were obtained from three independent experiments.

4. Conclusions

The fluorescence quenching capability of Hg$^{2+}$ on the TMPyP-dT$_{30}$ has been proposed, enabling a sensitive and selective Hg$^{2+}$ assay. The formation of the Hg-TMPyP-dT$_{30}$ ternary complex was characterized by various techniques. The mechanism inherent in the fluorescence quenching of TMPyP-dT$_{30}$ by Hg$^{2+}$ involved a synergistic effect. The detection limit of the proposed method was obtained as 1.3 nM. Experiments for tap water and river water demonstrated that the detection method was applicable for Hg$^{2+}$ determination in real samples. The Hg$^{2+}$ sensor based on oligonucleotide dT$_{30}$ enhanced TMPyP fluorescence was fast and low-cost, presenting a promising platform for practical Hg$^{2+}$ determination. In addition, this is the first time dT$_{30}$ enhanced TMPyP fluorescence intensity was used for Hg$^{2+}$ determination. The sensing protocol may provide useful information on the interaction among porphyrins, DNA and heavy metal ions.

Author Contributions: Conceptualization, D.W.; methodology, S.H.; investigation, D.W., Y.H. and S.H.; data curation, D.W. and Y.H.; writing—original draft preparation, D.W.; writing—review and editing, X.Y. and J.W.; supervision, X.Y. and J.W.; project administration, X.Y. and J.W.; funding acquisition, J.W.

Funding: The authors thank the National Natural Science Foundation of China (Nos. 21705166 and 21575166) for the financial support of this work.

Conflicts of Interest: The authors declare no conflicts of interest.
References

1. Biesaga, M.; Pyrzynska, K.; Trojanowicz, M. Porphyrins in analytical chemistry. A review. Talanta 2000, 51, 209–224. [CrossRef]

2. Zhang, L.; Lei, J.; Ma, F.; Ling, P.; Liu, J.; Ju, H. A porphyrin photosensitized metal-organic framework for cancer cell apoptosis and caspase responsive thanatosics. Chem. Commun. 2015, 51, 10831–10834. [CrossRef] [PubMed]

3. Pasternack, R.F.; Gibbs, E.J.; Villafranca, J.J. Interactions of porphyrins with nucleic acids. Biochemistry 1983, 22, 5409–5417. [CrossRef] [PubMed]

4. Pasternack, R.F.; Gibbs, E.J.; Gaudemer, A.; Antebi, A.; Bassner, S.; De Poy, L.; Turner, D.H.; Williams, A.; Laplace, F.; Lansard, M.H.; et al. Molecular complexes of nucleosides and nucleotides with a monomeric cationic porphyrin and some of its metal derivatives. J. Am. Chem. Soc. 1985, 107, 8179–8186. [CrossRef] [PubMed]

5. Kuroda, R.; Tanaka, H. DNA-porphyrin interactions probed by induced CD spectroscopy. J. Chem. Soc. Chem. Commun. 1994, 13, 1575–1576. [CrossRef]

6. Gaier, A.J.; Ghimire, S.; Fix, S.E.; McMillin, D.R. Internal versus external binding of cationic porphyrins to single-stranded DNA. Inorg. Chem. 2014, 53, 5467–5473. [CrossRef] [PubMed]

7. Kano, K.; Fukuda, K.; Wakami, H.; Nishiyabu, R.; Pasternack, R.F. Factors influencing self-aggregation tendencies of cationic porphyrins in aqueous solution. J. Am. Chem. Soc. 2000, 122, 7494–7502. [CrossRef]

8. Kubát, P.; Lang, K.; Anzenbacher, P., Jr.; Jursíksová, K.; Král, V.; Ehrenberg, B. Interaction of novel cationic meso-tetraphenylporphyrins in the ground and excited states with DNA and nucleotides. J. Chem. Soc. Perkin. Trans. 2000, 6, 933–941. [CrossRef]

9. Guliaev, A.B.; Leonits, N.B. Cationic 5,10,15,20-tetrakis(N-methylpyridinium-4-yl)porphyrin fully intercalates at 5′-CG-3′ steps of duplex DNA in solution. Biochemistry 1999, 38, 15425–15437. [CrossRef] [PubMed]

10. Novy, J.; Urbanova, M. Vibrational and electronic circular dichroism study of the interactions of cationic porphyrins with (dG-dC)10 and (dA-dT)10. Biopolymers 2007, 85, 349–358. [CrossRef] [PubMed]

11. Ohyama, T.; Mita, H.; Yamamoto, Y. Binding of 5,10,15,20-tetrakis(N-methylpyridinium-4-yl)-21H,23H-porphyrin to an AT-rich region of a duplex DNA. Biophys. Chem. 2005, 113, 53–59. [CrossRef] [PubMed]

12. Lee, Y.; Kim, J.; Cho, T.; Song, R.; Kim, S.K. Binding of meso-Tetrakis(-methylpyridinium-4-yl)porphyrin to triplex oligonucleotides: Evidence for the porphyrin stacking in the major groove. J. Am. Chem. Soc. 2003, 125, 8106–8107. [CrossRef] [PubMed]

13. Lubitz, I.; Borovnik, N.; Kottiyar, A. Interaction of monomolecular G4-DNA nanowires with TMPyP: Evidence for intercalation. Biochemistry 2007, 46, 12925–12929. [CrossRef] [PubMed]

14. Zhang, L.; Zhu, J.; Ai, J.; Zhou, Z.; Jia, X.; Wang, E. Label-free G-quadruplex-specific fluorescent probe for sensitive detection. Biosens. Bioelectron. 2013, 39, 268–273. [CrossRef] [PubMed]

15. Gaier, A.J.; McMillin, D.R. Binding studies of G-quadruplex DNA and porphyrins: Cu(T4) vs sterically friendly Cu(TD4). Inorg. Chem. 2015, 54, 4504–4511. [CrossRef] [PubMed]

16. Dave, N.; Chan, M.Y.; Huang, P.J.; Smith, B.D.; Liu, J. Regenerable DNA-functionalized hydrogels for ultrasensitive, instrument-free mercury(II) detection and removal in Water. J. Am. Chem. Soc. 2010, 132, 12668–12673. [CrossRef] [PubMed]

17. McClure, D.S. Spin-orbit interaction in aromatic molecules. J. Chem. Phys. 1952, 20, 682–686. [CrossRef]

18. Wu, G.W.; He, S.B.; Peng, H.P.; Deng, H.H.; Liu, A.L.; Lin, X.H.; Xia, X.H.; Chen, W. Citrate-capped platinum nanoparticle as a smart probe for ultrasensitive mercury(II) detection and removal in Water. Inorg. Chem. 2015, 54, 4504–4511. [CrossRef] [PubMed]

19. Srinivasan, K.; Subramanian, K.; Murugan, K.; Dinakaran, K. Sensitive fluorescence detection of mercury(II) in aqueous solution by the fluorescence quenching effect of MoS2 with DNA functionalized carbon dots. Analyst 2016, 141, 6344–6352. [CrossRef] [PubMed]

20. Jasuja, R.; Jameso, D.M.; Nishijo, C.K.; Larsen, R.W. Singlet excited state dynamics of tetrakis(4-N-methylpyridyl)porphine associated with DNA nucleotides. J. Phys. Chem. B 1997, 101, 1444–1450. [CrossRef]

21. Zhu, K.; Hu, X.; Ge, Q.; Sun, Q. Fluorescent recognition of deoxyribonucleic acids by a quantum dot/meso-tetrakis(N-methylpyridinium-4-yl)porphyrin complex based on a photo induced electron-transfer mechanism. Anal. Chim. Acta 2014, 812, 199–205. [CrossRef] [PubMed]
22. Paulo, P.R.; Costa, S.B. Interactions in noncovalent PAMAM/TMPyP systems studied by fluorescence spectroscopy. *J. Phys. Chem. B* 2005, 109, 13928–13940. [CrossRef] [PubMed]
23. Yang, R.; Li, K.; Wang, K.; Zhao, F.; Li, N.; Liu, F. Porphyrin assembly on β-cyclodextrin for selective sensing and detection of a zinc ion based on the dual emission fluorescence ratio. *Anal. Chem.* 2003, 75, 612–621. [CrossRef] [PubMed]
24. Schulman, S.G. (Ed.) *Molecular Luminescence Spectroscopy. Methods and Applications, Part I*; Wiley: New York, NY, USA, 1988.
25. Choi, J.K.; Sargsyan, G.; Olive, A.M.; Balaz, M. Highly sensitive and selective spectroscopic detection of mercury(II) in water by using pyridylporphyrin-DNA conjugates. *Chem. Eur. J.* 2013, 19, 2515–2522. [CrossRef] [PubMed]
26. Delmarre, D.; Méallet, R.; Bied-Charreton, C.; Pansu, R.B. Heavy metal ions detection in solution, in Sol-gel and with grafted porphyrin monolayers. *J. Photochem. Photobiol. A* 1999, 124, 23–28. [CrossRef]
27. Xu, J.; Wu, J.; Zong, C.; Ju, H.; Yan, F. Manganese porphyrin dsDNA complex: A mimicking enzyme for highly efficient bioanalysis. *Anal. Chem.* 2013, 85, 3374–3379. [CrossRef] [PubMed]
28. Nový, J.; Urbanová, M.; Volka, K. Vibrational and electronic circular dichroism and absorption spectral study of the DNA-5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin interaction. *J. Mol. Struct.* 2005, 748, 17–25. [CrossRef]
29. Guo, L.; Hu, H.; Sun, R.; Chen, G. Highly sensitive fluorescent sensor for mercury ion based on photoinduced charge transfer between fluorophore and π-stacked T–Hg(II)-T base pairs. *Talanta* 2009, 79, 775–779. [CrossRef] [PubMed]
30. Torigoe, H.; Ono, A.; Kozasa, T. HgII ion specifically binds with T:T mismatched base pair in duplex DNA. *Chem. Eur. J.* 2010, 16, 13218–13225. [CrossRef] [PubMed]
31. Wang, Y.; Pan, F.; Zhang, Y.; Peng, F.; Huang, Z.; Zhang, W.; Zhao, W. A dual-mode turn-on fluorescent BODIPY-based probe for visualization of mercury ions in living cells. *Analyst* 2016, 141, 4789–4795. [CrossRef] [PubMed]
32. Chen, L.; Zhao, Y.; Wang, Y.; Zhang, Y.; Liu, Y.; Han, X.X.; Zhao, B.; Yang, J. Mercury species induced frequency-shift of molecular orientational transformation based on SERS. *Analyst* 2016, 141, 4782–4788. [CrossRef] [PubMed]
33. Chen, G.H.; Chen, W.Y.; Yen, Y.C.; Wang, C.W.; Chang, H.T.; Chen, C.F. Detection of mercury(II) ions using colorimetric gold nanoparticles on paper-based analytical devices. *Anal. Chem.* 2014, 86, 6843–6849. [CrossRef] [PubMed]
34. Wei, Q.; Nagi, R.; Sadeghi, K.; Steve, F.; Yan, E.; Ki, S.J.; Caire, R.; Tseng, D.; Ozcan, A. Detection and spatial mapping of mercury contamination in water samples using a Smart-Phone. *ACS Nano* 2014, 8, 1121–1129. [CrossRef] [PubMed]
35. Zhang, H.; Xia, Y. Ratiometry, wavelength, and intensity: Triple signal readout for colorimetric sensing of mercury ions by plasmonic Cu2-xSe nanoparticles. *ACS Sens.* 2016, 1, 384–391. [CrossRef]
36. Zhang, J.; Tian, J.; He, Y.; Zhao, Y.; Zhao, S. A K+-mediated G-quadruplex formation enhancement fluorescence polarization system based on quantum dots for detection of Hg2+ and biothiols. *Chem. Commun.* 2014, 50, 2049–2051. [CrossRef] [PubMed]
37. Huang, P.J.; Wang, F.; Liu, J. Cleavable molecular beacon for Hg2+ detection based on phosphorothioate RNA modifications. *Anal. Chem.* 2015, 87, 6890–6895. [CrossRef] [PubMed]
38. Aragay, G.; Pons, J.; Merkoçi, A. Recent trends in macro-, micro-, and nanomaterial-based tools and strategies for heavy-metal detection. *Chem. Rev.* 2011, 111, 3433–3458. [CrossRef] [PubMed]
39. Lemon, C.M.; Brothers, P.J.; Boitrel, B. Porphyrin complexes of the period 6 main group and late transition metals. *Dalton Trans.* 2011, 40, 6591–6609. [CrossRef] [PubMed]