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Adjusting the Structure of a Peptide Nucleic Acid (PNA) Molecular Beacon and Promoting Its DNA Detection by a Hybrid with Quencher-Modified DNA

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Abstract: In this study, we performed an elaborate adjustment of the structure of peptide nucleic acid (PNA) molecular beacons as probes for detecting nucleic acids. We synthesized the PNA beacons with various numbers of Glu, Lys, and dabcy1 (Dab) quenchers in them, and we investigated their fluorescence changes ($F_{1/1}/F_0$) with and without full-match DNA. As the numbers of Glu/Lys or Dab increased, the $F_{1/1}/F_0$ tended to decrease. Among the different beacons, the PNA beacon with one Glu and one Lys (**P1Q1**) showed the largest $F_{1/1}/F_0$. On the other hand, a relatively large $F_{1/1}/F_0$ was obtained when the number of Glu/Lys and the number of Dab were the same, and the balance between the numbers of Glu/Lys and Dab seemed to affect the $F_{1/1}/F_0$. We also investigated the DNA detection by the prehybrid of **P1Q1**, which consists of the T790M base sequence, [**P1Q1(T790M)**], with quencher-modified DNA (Q-DNA). We examined the DNA detection with single-base mismatch by **P1Q1(T790M)**, and we clarified that there was difficulty in detecting the sequence with **P1Q1** alone, but that the sequence was successfully detected by the prehybrid of **P1Q1** with the Q-DNA.

Keywords: peptide nucleic acid; DNA; molecular beacon; fluorescence



Citation: Shigeto, H.; Kishi, T.; Ishii, K.; Ohtsuki, T.; Yamamura, S.; Kitamatsu, M. Adjusting the Structure of a Peptide Nucleic Acid (PNA) Molecular Beacon and Promoting Its DNA Detection by a Hybrid with Quencher-Modified DNA. *Processes* **2022**, *10*, 722. <https://doi.org/10.3390/pr10040722>

Academic Editors: Kenji Usui and Kin-ya Tomizaki

Received: 18 March 2022

Accepted: 6 April 2022

Published: 8 April 2022

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1. Introduction

Peptide nucleic acid (PNA) [1] is a nucleic acid surrogate that forms a stable-base sequence-specific hybrid with DNA and RNA [2]. Since PNA consists of an uncharged amide backbone, PNA/DNA and PNA/RNA hybrids do not exhibit electrostatic repulsion, in contrast to DNA/DNA and DNA/RNA hybrids, which results in greater stability. Therefore, it is expected that PNA can be used as a probe for detecting nucleic acids, and colorimetric detection using dye [3–10] and mass-based [11,12], visual [13], electrochemical [11,14–20], and optical [18,21] detection, among others, has been reported. Furthermore, the detection of nucleic acids on the basis of fluorescence by conjugating PNA and fluorescent dyes is plausible as a highly sensitive detection method, and numerous studies that use these conjugates as probes to detect DNA and RNA have been reported [22].

Among these reported approaches, PNA molecular beacons (PNA beacons), in which the PNA is modified by a fluorescent dye and a quencher, are useful as illuminating probes for detecting DNA and RNA. A typical PNA beacon contains one fluorescent dye (Fam) and one Dab quencher at both termini, and it contains, adjacent to these groups, one negatively (Glu) and one positively (Lys) charged amino acid residue [23–32]. Before the PNA beacon hybridizes with DNA or RNA, the Glu and the Lys are close to each other because of the intramolecular electrostatic interaction; as a result, the fluorescent dye and the quencher are

also close to each other, and the PNA beacon is quenched. On the other hand, as the PNA beacon forms a hybrid with the target DNA and RNA, thereby relieving the interaction between the Glu and the Lys, the fluorescent dye and the quencher become more distant from each other, and the PNA beacon emits fluorescence.

In this work, we consider that the adjustment of the intramolecular electrostatic interaction by varying the numbers of Glu and Lys in the PNA beacon is important in order to obtain a better performance of the beacon. However, to the best of our knowledge, no discussion has yet been conducted on the numbers of Glu/Lys in this context. Therefore, we prepared PNA beacons that contain various numbers of Glu/Lys, while also varying the number of Dab quenchers, in order to obtain a PNA beacon that fluoresces more effectively when hybridized with nucleic acid. We also assessed the fluorescence characteristics in this study. In addition, we recently reported a system in which a hybrid of Fam-modified PNA (Fl-PNA) and Dab-modified DNA (Q-DNA) detected a target nucleic acid by emitting light from the Fl-PNA via strand exchange with the target nucleic acid [33–37]. In this report, we describe that the use of the PNA beacon, instead of the Fl-PNA, further improves nucleic acid detection.

2. Materials and Methods

2.1. Materials

9-Fluorenylmethyloxycarbonyl group (Fmoc)-derivatized amino acids, Fmoc-derivatized super acid labile-poly(ethylene)glycol (Fmoc-NH-SAL-PEG) resin, piperidine, *O*-(1H-benzotriazol-1-yl)-*N,N,N'*, *N'*-tetramethyluronium hexafluorophosphate (HBTU), *N*-methylmorpholine (NMM), trifluoroacetic acid (TFA), and triisopropylsilane (TIPS) were purchased from Watanabe Chemicals (Hiroshima, Japan). Fmoc-derivatized PNA monomers [Fmoc-A(Bhoc)-OH, Fmoc-T-OH, Fmoc-G(Bhoc)-OH, and Fmoc-C(Bhoc)-OH] were purchased from Panagene (Daejeon, Korea). Fluorescent dye (5(6)-carboxyfluorescein (Fam)), and Fmoc-Lys(dabcy)-OH as a Dab quencher, were purchased from AAT Bioquest (Sunnyvale, CA, USA). Fmoc-derivatized ethylene glycol linker, containing six ethylene glycol units ((Fmoc-amino)-PEG6-carboxylic acid), was purchased from the Tokyo Chemical Industry (Tokyo, Japan). *N,N'*-Dimethylformamide (DMF), *N*-methyl-2-pyrrolidinone (NMP), diethyl ether, and acetonitrile were purchased from the FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Phosphate-buffered saline (PBS) (100 mM; pH: 7.0) was purchased from Nacalai Tesque (Kyoto, Japan). DNA oligomers were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Peptide Synthesis

All PNA beacons [**PnQms**, **P1Q1(T790M)**, **P1Q1(L858R)**, and **P1Q1(exon19del)**; Figure 1] were synthesized on Fmoc-NH-SAL-PEG resin, containing 7.2 μ mol Fmoc on its surface, by using standard Fmoc protection chemistry. The deprotection and coupling processes were carried out at room temperature. The Fmoc group was removed by using 20% piperidine in DMF for 7 min. Each coupling process included: 4 parts Fmoc-derivatized amino acids; Fmoc-derivatized PNA monomers; an Fmoc-derivatized ethylene glycol linker; a Fam or Dab quencher; 3.6 parts HBTU; and 11.5 90 min at room temperature. Crude peptides were precipitated in diethyl ether and were washed twice with diethyl ether until a neutral pH was reached. Peptides were then dried by parts, and NMM were dissolved in DMF/NMP and were added to the resin. The reaction mixture was then shaken for 45 min. Peptides on the resin were by treatment with 95:2.5:2.5 (*v/v*) TFA/TIPS/water, and they were dissolved in 50:50 (*v/v*) 0.1% aqueous TFA in water/acetonitrile. Peptides were purified using reverse-phase high-pressure liquid chromatography (HPLC) on a C18 preparative column (Cadenza 5CD-C18; Imtakt, Kyoto, Japan). The final product was identified by using matrix-assisted laser desorption/ionization–time-of-flight (MALDI-ToF) mass spectrometry (Shimadzu AXIMA Confidence) (Supplementary Material, Figures S1–S17) and HPLC on a C18 analytical column (Cadenza CD-C18; Imtakt) (Supplementary Material, Figures S18–S34).

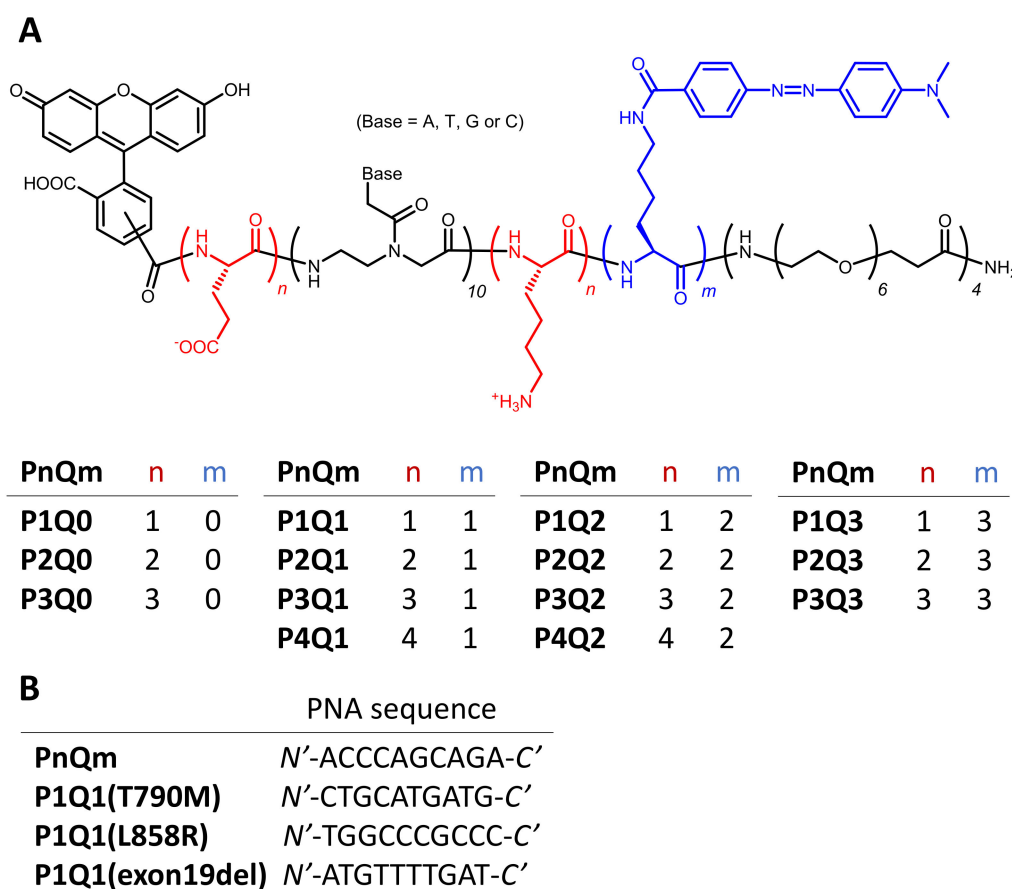


Figure 1. (A) Chemical structures of 14 PNA beacons (**PnQm** ($n = 1-4$ and $m = 0-3$)) and three **P1Q1**s with different PNA sequences: **P1Q1(T790M)**, **P1Q1(L858R)**, and **P1Q1(exon19del)**. The N-terminal and near C-terminal in the PNA beacon were modified with Fam and Dab quenchers, respectively. These PNA beacons were modified with four ethylene glycol linkers in the C-terminal to improve the water solubility. (B) The PNA sequences of **PnQms**, **P1Q1(T790M)**, **P1Q1(L858R)**, and **P1Q1(exon19del)**.

2.3. Melting Curves of Mixtures of PNA Beacon and DNA

Molar concentrations of PNA beacons and DNAs were estimated from the absorbance at 260 nm and were measured by using a UV-vis spectrometer (JASCO V-560) with molar extinction coefficients of the nucleobases. The UV melting curves of the equimolar mixtures of **P1Q1** or **P4Q1**, with and without full-match DNA (fmDNA: 5'-TCTGCTGGGT-3') or scrambled DNA (scrDNA: 5'-CGTGGTTCTG-3'), in aqueous buffer (100 mM PBS; pH: 7.0), were measured by using a Shimadzu TMSPC-8 Tm Analysis System that was equipped with a UV-visible spectrometer (UV-1700) and an eight-position Peltier temperature controller. Each concentration of the PNA beacons was 5.0 μ M, and the quartz cell length was 1 cm. The melting curves were recorded with the cooling of the solution by 0.5 $^{\circ}$ C/0.5 min, from 80 to 10 $^{\circ}$ C, while measuring the absorbance at 260 nm. The observed absorbance was normalized to that at 80 $^{\circ}$ C. The melting temperature, at which 50% of the strands remained hybridized (T_m), was obtained by using a TMSPC-8 with Tm analysis software.

2.4. Fluorescence Titration Curves of PNA Beacon and DNA

The fluorescence spectra of the 500 nM **PnQm** and DNA (fmDNA and scrDNA) mixtures, in aqueous buffer (100 mM PBS; pH: 7.0) at 25 $^{\circ}$ C, were measured at an excitation wavelength of 495 nm, and at emission wavelengths from 500 to 700 nm, by using a JASCO FP-8200 fluorescence spectrometer and a 1 cm quartz cell. In the case of the titration curves, the concentration of **PnQm** was maintained at 500 nM, while the concentrations of DNA

were varied in order to create **PnQm**/DNA ratios of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, or 2.0. The fluorescence spectra were measured after the **PnQm** was incubated with DNA for 15 min. The fluorescence titration curves were recorded on the basis of the fluorescence intensities at 525 nm. The assessment of the DNA detection by **PnQm** was conducted by measuring the ratio (F/F_0) of the fluorescence intensity (at 525 nm) of the PNA beacon without DNA (F_0) to that of the PNA beacon with DNA (F). In the case of the fluorescence spectra of **P1Q1(T790M)**, **P1Q1(L858R)**, and **P1Q1(exon19del)** with DNA, we used T790M: 5'-CATCATGCAG-3' L858R: 5'-GGGCGGGCCA-3', and exon19del: 5'-ATCAAAACAT-3' as the DNAs.

2.5. Relative Fluorescence of PNA Beacon and PNA Beacon/Q-DNA Hybrid with Target DNA

P1Q1(T790M) and 8-mer DNA containing a Dab quencher at the 3'-end (Q-DNA; 5'-TCATGCAG-Dab-3') were mixed in PBS (each 500 nM). The mixture was incubated at 95 °C for 5 min and was then slowly cooled to 25 °C to form the hybrid. Then, the fluorescence spectra of the **P1Q1(T790M)**/Q-DNA hybrid were measured at 37 °C by using an excitation wavelength of 480 nm, and emission wavelengths from 500 to 700 nm. Next, a complementary DNA (5'-CAC CGT GCA ACT CAT CAT GCA GCT CAT GCC CTT C-3') and one-base-mismatched DNA (5'-CAC CGT GCA ACT CAT CAC GCA GCT CAT GCC CTT C-3') were mixed at 1:1 with the **P1Q1(T790M)**/Q-DNA hybrid, and fluorescence spectra were measured at 37 °C. Each concentration of DNA and the hybrid was 500 nM. The fluorescence spectrum of the **P1Q1(T790M)**, instead of the **P1Q1(T790M)**/Q-DNA hybrid, was also measured in the same manner as described above. The DNA detection by **P1Q1(T790M)** and **P1Q1(T790M)**/Q-DNA was assessed by the same protocol, as described in Section 2.4.

3. Results

3.1. Hybrid Formation of DNA with PNA Beacon and Fluorescence Detection of DNA by PNA Beacon

To confirm that the synthesized PNA beacons (**PnQms**) formed hybrids with the DNA, we measured the UV melting curves of a 1:1 mixture of the **P1Q1** and DNAs (left panel in Figure 2). A clear sigmoid curve was observed for the mixture of **P1Q1** and full-match DNA (fmDNA), which indicates that they formed a hybrid (red-filled circles: $T_m = 65$ °C). On the other hand, for **P1Q1** alone, and for **P1Q1** with scrambled DNA (scrDNA), the absorption changed linearly under the applied conditions, which indicates that a hybrid did not form (blue- and black-filled circles, respectively). The UV melting curve of a 1:1 mixture of **P4Q1** with and without DNAs also showed similar results (right panel in Figure 2; $T_m = 66$ °C for **P4Q1**/fmDNA). From these results, we confirmed that the PNA beacons and DNA sufficiently form a sequence-specific hybrid under the conditions of 25 °C and 37 °C, and we subsequently performed the fluorescence assessment.

To confirm that **PnQms** detected the target DNA, we measured the fluorescence spectra of **P1Q1** (as a typical PNA beacon) and **P1Q0** (not modified by a Dab quencher), as a control, with and without an equimolar amount of fmDNA. As shown in Figure 3, **P1Q0** showed fluorescence that was derived from a Fam group at 520 nm (black solid line); however, the fluorescence intensity was less than that of Fam [5(6)-carboxyfluorescein] (green solid line). This indicates that the peptide chain affects the fluorescence properties of the Fam group. The intensity of **P1Q0** with fmDNA (black broken line) was lower than that of **P1Q0**. On the other hand, **P1Q1** showed almost no fluorescence under these conditions (red solid line), whereas, in the case of **P1Q1** with fmDNA, the fluorescence was observed at 530 nm (red broken line). This fluorescence indicates that, when **P1Q1** forms a hybrid with fmDNA, the Fam group moves away from the Dab quencher in the PNA beacon, and the fluorescence that is derived from the Fam group is reactivated. In other words, **P1Q1** is well folded in the molecule before forming the hybrid with fmDNA, and it is suggested that Dab quenches the fluorescence of the Fam group. The intensity of **P1Q1** with fmDNA

cannot be restored to that of **P1Q0**, which indicates that the chain length of the PNA beacon, which is 10-mer, does not keep the Fam group far enough away from the Dab quencher.

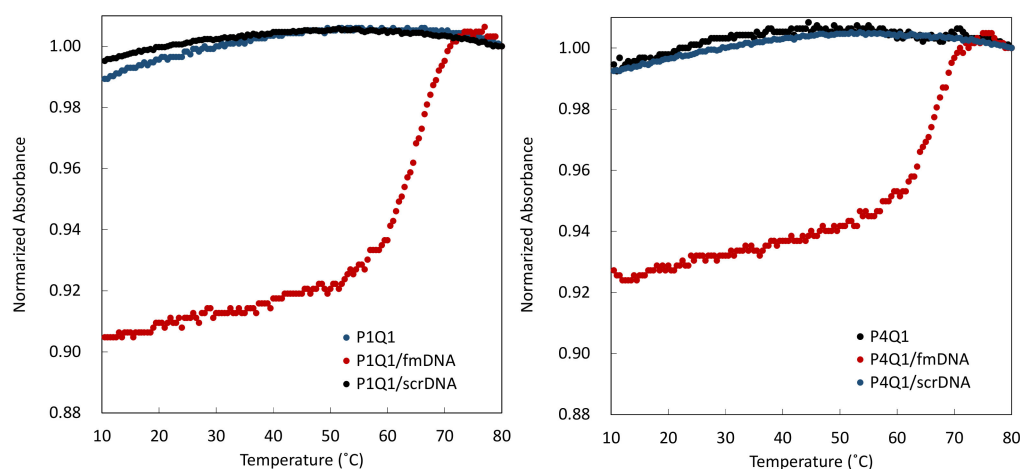


Figure 2. Temperature dependences of absorption intensity at 260 nm for: (left) 1:1 mixtures of **P1Q1**/fmDNA (red-filled circles) and **P1Q1**/scrDNA (black-filled circles), and **P1Q1** alone (blue-filled circles); and (right) 1:1 mixtures of **P4Q1**/fmDNA (red-filled circles) and **P4Q1**/scrDNA (black-filled circles), and **P4Q1** alone (blue-filled circles).

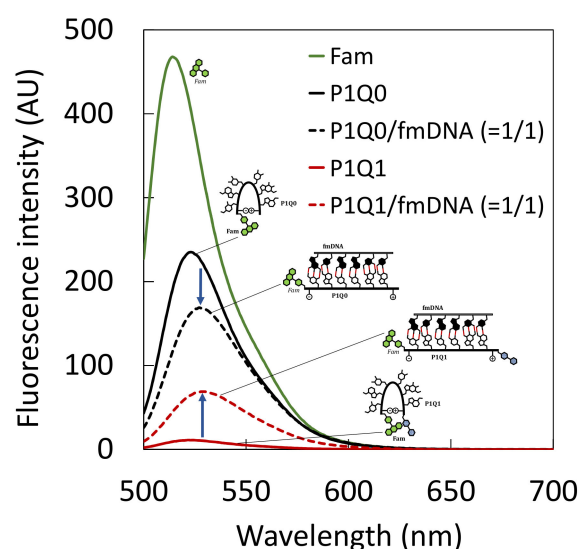


Figure 3. Fluorescence spectra of 500 nM **P1Q1** and **P1Q0** (red and black solid lines, respectively), and 1:1 mixtures of **P1Q1**/fmDNA and **P1Q0**/fmDNA (red and black broken lines, respectively). Fam was also observed as a control (green solid line).

To examine the fluorescence characteristics of the **P1Q1** with DNAs, we measured fluorescence spectra for various concentrations of DNAs and created their titration curves (Figure 4). As shown in the left panel in Figure 4A, the fluorescence spectra of **P1Q1** shows that the fluorescence intensity at 525 nm increased as the fmDNA was gradually added. On the other hand, the addition of scrDNA, as shown in the right panel in Figure 4A, shows almost no change at 525 nm. This indicates that the PNA beacon forms a sequence-specific hybrid with the DNAs. Next, we plotted the fluorescence intensity (F/F_0) at 525 nm for each DNA/**P1Q1** ratio on the basis of the fluorescence spectra in Figure 4A,B. As the fmDNA was added from a 0 to 1 equivalent, the F/F_0 increased linearly, and, above a 1 equivalent, the F/F_0 did not change (blue-filled circles). On the other hand, when scrDNA was added, the **P1Q1** hardly emitted fluorescence under these conditions (red-filled circles). These results show that **P1Q1** forms a 1:1 hybrid with full-match DNA.

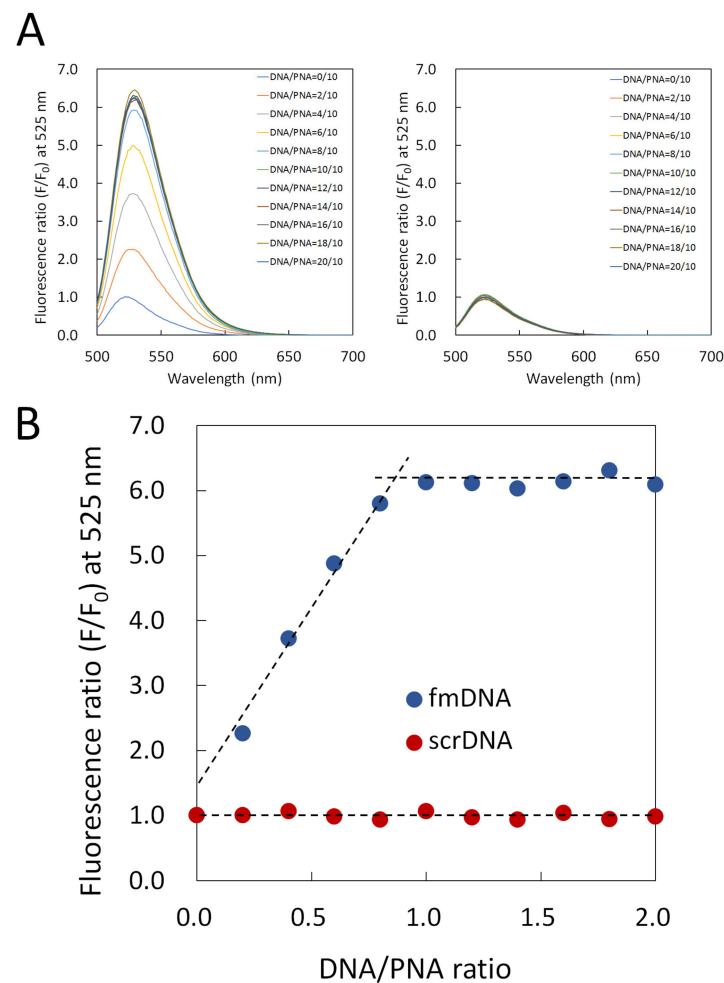


Figure 4. (A) Fluorescence spectra of 500 nM **P1Q1** with various concentrations of fmDNA (left) and scrDNA (right). (B) Titration curves of fluorescence intensities at 525 nm for **P1Q1**/fmDNA (blue-filled circles) and **P1Q1**/scrDNA (red-filled circles).

3.2. Elaborate Adjustment of PnQms for Fluorescence Detection of DNA

The structure of the PNA beacon, which is reported by Kuhn et al. (**P1Q1** in this study), is used as a typical PNA beacon [23]. We are interested in whether this PNA beacon is the optimal structure for detecting nucleic acids. Therefore, we used **PnQms**, in which the numbers of Glu/Lys and Dab quenchers in the PNA beacon were changed, and we assessed these levels of DNA detection by the fluorescence titration curves for the DNA, in the same manner as in Figure 4. **PnQ0**, which does not contain the Dab quencher, showed a slight decrease in fluorescence upon the addition of fmDNA, despite varying the number of Glu/Lys from 1 to 3, which indicates that it is difficult for **PnQ0** to detect DNA by fluorescence (Figure 5A and Supplementary Material, Figures S35–S37). On the other hand, for **PnQ1**, a clear 1:1 correspondence of the increase in the fluorescence upon the addition of fmDNA was observed, and the response was further influenced by the number of Glu/Lys (Figure 5B and Supplementary Material, Figures S38–S41). The $F_{1/1}/F_0$ value, which is the fluorescence ratio of the 1:1 mixtures of the PNA beacon with fmDNA to the PNA beacon alone, showed higher fluorescence levels in the order of: **P1Q1** > **P2Q1** = **P3Q1** > **P4Q1**. It was confirmed that the $F_{1/1}/F_0$ values of **PnQ2** and **PnQ3** also depend on the number of Glu/Lys: **P2Q2** $\approx$ **P3Q2** > **P1Q2** $\approx$ **P4Q2** (Figure 5C and Supplementary Material, Figures S42–S45) and **P3Q3** > **P1Q3** = **P2Q3** (Figure 5D and Supplementary Material, Figures S46–S48).

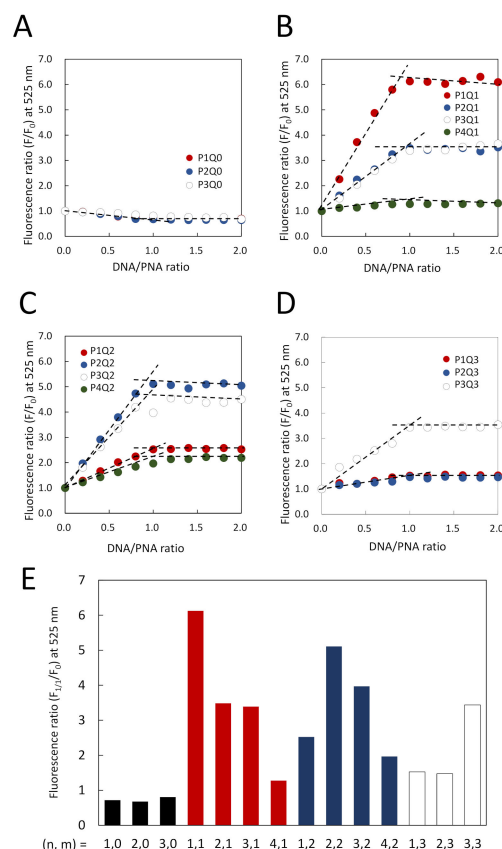


Figure 5. Titration curves of fluorescence intensities at 525 nm for: (A) **PnQ0** ($n = 1-3$); (B) **PnQ1** ($n = 1-4$); (C) **PnQ2** ($n = 1-4$); and (D) **PnQ3** ($n = 1-3$), with fmDNA. (E) Fluorescence ratios ($F_{1/1}/F_0$) of the 1:1 mixtures of **PnQm** and fmDNA.

As a summary of Figure 5A–D, each $F_{1/1}/F_0$ of **PnQms** is shown in Figure 5E. This indicates that the numbers of Glu/Lys (n) and Dab quenchers (m) show a relatively large $F_{1/1}/F_0$ when $n = 1$ and $m = 1$ ($F_{1/1}/F_0 = 6.1$). When the number of m is 3, the $F_{1/1}/F_0$ level decreased, which indicates that the quenching effect of Dab against the Fam group was too strong. Regarding the number of n , the $F_{1/1}/F_0$ level tended to decrease from a smaller number to a larger number. We assume that, as the number of Glu/Lys increases, the electrostatic interaction between Glu and Lys may inhibit the hybrid formation of PNA and DNA. On the other hand, a relatively high $F_{1/1}/F_0$ level was observed when the numbers, n and m , were the same ($F_{1/1}/F_0 = 6.1, 5.1$, and 3.4 for $n, m = 1.1, 2.2$, and 3.3 , respectively). This shows that not only the numbers of n and m , but also the balance of the numbers of n and m , affect the $F_{1/1}/F_0$ value. Overall, we determined that, among these PNA beacons, the PNA beacon with the highest relative fluorescence is **P1Q1**. This PNA beacon is consistent with the PNA beacon that is reported by Kuhn et al.

To investigate whether **P1Q1** detects various DNA sequences, we used three **P1Q1s** with different PNA sequences: **P1Q1(T790M)**: N' -CTGCATGATG-C'; **P1Q1(L858R)**: N' -TGGCCCGCCC-C'; and **P1Q1(exon19del)**: N' -ATGTTTGTGAT-C'. The $F_{1/1}/F_0$ ratio was estimated from the fluorescence spectra before and after the addition of equivalent amounts of full-match DNAs (T790M: 5'-CATCATGCAG-3'; L858R: 5'-GGGCGGGCCA-3'; and exon19del: 5'-ATCAAAACAT-3') to each of the **P1Q1s**. As shown in Figure 6 and in the Supplementary Material, Figures S49–S51, the $F_{1/1}/F_0$ values of **P1Q1(T790M)**, **P1Q1(L858R)**, and **P1Q1(exon19del)** for full-match DNA were 2.5, 4.7, and 7.2, respectively, whereas these values for noncomplementary DNA were about 1.0–1.2. Among these sequences, the higher $F_{1/1}/F_0$ of **P1Q1(exon19del)** may be influenced by the self-complementary sequences of the two nucleobases at both ends. These indicate that the series of **P1Q1** recognizes full-match DNA, although this depends on the PNA sequence.

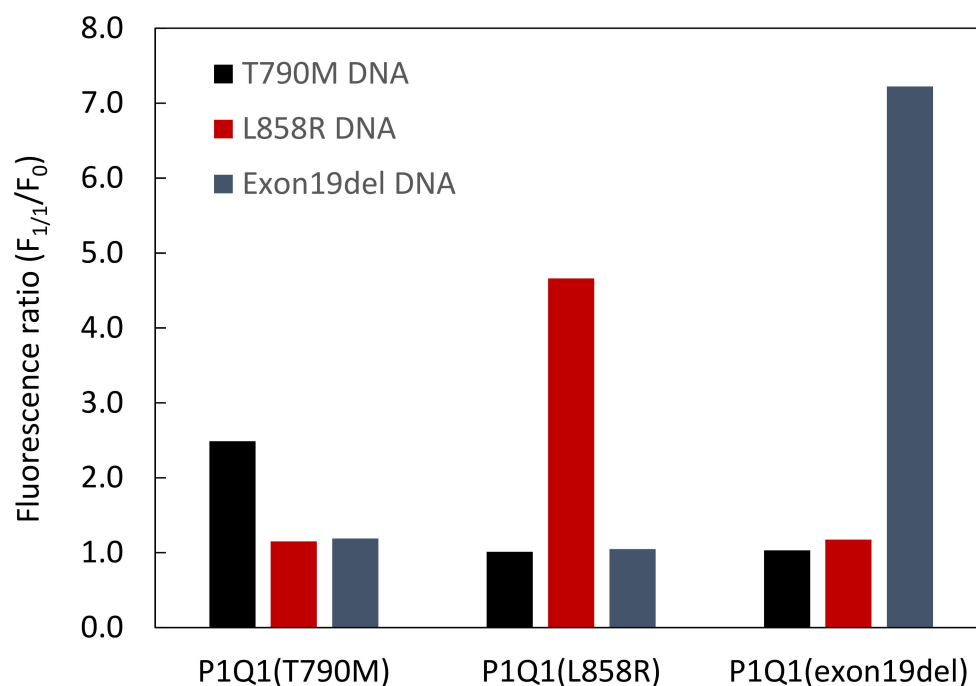


Figure 6. Fluorescence ratios ($F_{1/1}/F_0$) of the 1:1 mixtures of **P1Q1**s with different PNA sequences and DNAs.

3.3. Comparison of Fluorescence Detection for Target DNA of PNA Beacon and PNA Beacon/Quencher-Modified DNA Hybrid

In previous studies, we reported that a probe that prehybridized Fam-modified PNA (Fl-PNA) with a Dab quencher-modified DNA (Q-DNA) successfully detected target DNA [35,36], and that a probe that prehybridized the PNA beacon with Q-DNA detected target miRNA [37]. To investigate whether the prehybridized Q-DNA and PNA beacon in this study detect target DNA more effectively than the PNA beacon alone, we assessed the $F_{1/1}/F_0$ value of the hybrid of **P1Q1(T790M)** with Q-DNA for target DNA (Figure 7 and Supplementary Material, Figure S52). The values of **P1Q1(T790M)** mixed with one-base-mismatched T790M DNA and **P1Q1(T790M)** alone were almost 1.0, whereas the value of **P1Q1(T790M)** mixed with full-match T790M DNA was 3.0. This was almost the same as the value shown by **P1Q1(T790M)** to T790M DNA in Figure 6 ($F_{1/1}/F_0 = 2.5$). In other words, **P1Q1(T790M)** detected the target DNA, but the recognition of the PNA beacon for DNA was not sufficient. On the other hand, the value of the **P1Q1(T790M)/Q-DNA** hybrid mixed with the one-base-mismatched DNA, and the value of the hybrid only, were almost 1.0, whereas the value of the hybrid mixed with the complementary DNA was 8.0. In a previous study, when the complementary T790M DNA was detected with the Fl-PNA/Q-DNA hybrid, the $F_{1/1}/F_0$ value was 1.5–2.0 [35]. These results indicate that the PNA beacon/Q-DNA hybrid detects the target DNA more effectively than the Fl-PNA/Q-DNA hybrid or the PNA beacon alone. From these results, we demonstrated that a synergistic or additive effect by the Dab quenchers contained in the PNA beacon and the Q-DNA was effectively exerted on the fluorescence detection of DNA in this study.

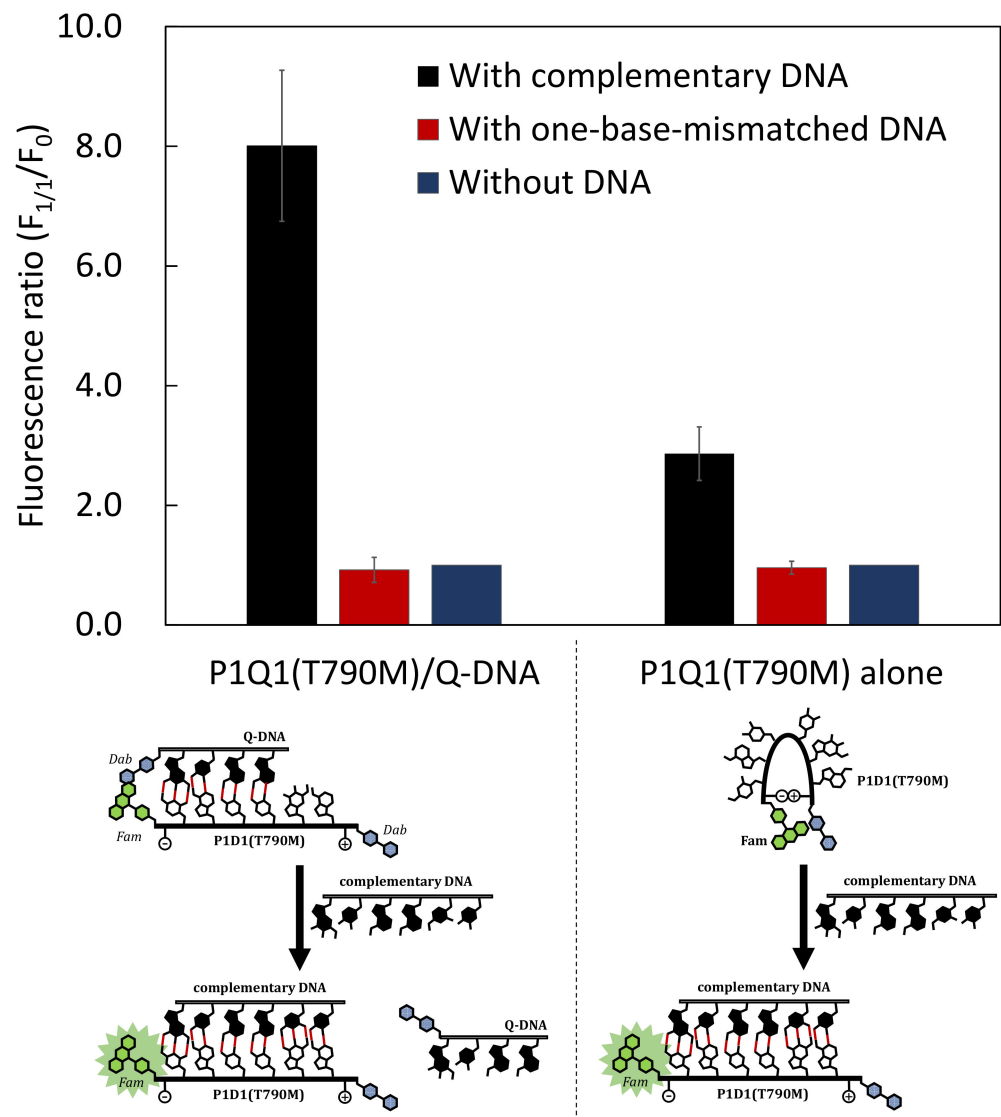


Figure 7. Fluorescence ratios ($F_{1/1}/F_0$) of 1:1 mixtures of **P1Q1(T790M)/Q-DNA** hybrid with DNAs, and of **P1Q1(T790)** with DNAs.

4. Conclusions

In this study, we optimized the number of Glu/Lys and the number of Dab quenchers in the PNA beacons (**PnQms**). As a result, we found that the structure of the conventional PNA beacon, **P1Q1**, is the best for the fluorescence detection of target nucleic acids. In other words, it was not necessary to increase the number of Glu/Lys and the number of Dab quenchers to improve the DNA detection of PNA beacons. However, we also obtained new findings that suggest that the balance between the number of Glu/Lys and the number of Dab quenchers affects the DNA detection of PNA beacons. This result is expected to be valuable to the optimization of the structures of other versions of PNA beacons. **P1Q1s** were shown to achieve detection, depending on the sequence of the target DNAs, but the hybrid of **P1Q1**, with quencher-modified DNA, conferred a more effective detection to the PNA sequences, with a relatively low $F_{1/1}/F_0$ value. This indicates that the combination of this PNA beacon and the PNA beacon/quencher-modified DNA hybrid probe is effective when detecting multiple DNA sequences. In the future, we will investigate by using a longer PNA than the PNA (10-mer) that was used in this study in order to develop the PNA beacon that targets RNA, and we plan to use the hybrid probe to detect nucleic acids in cells.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/pr10040722/s1>, Supplementary Material, Figures S1–S17: MALDI-ToF mass spectra of all peptides; Supplementary Material, Figures S18–S34: HPLC chromatograms of all peptides; Supplementary Material, Figures S35–S52: Fluorescence spectra of all peptides with DNAs.

Author Contributions: Conceptualization, T.O.; methodology, T.O. and S.Y.; software, M.K.; validation, M.K.; formal analysis, H.S., T.K. and K.I.; investigation, H.S., T.K. and K.I.; resources, M.K. and S.Y.; data curation, M.K. and H.S.; writing—original draft preparation, M.K.; writing—review and editing, T.O., S.Y. and H.S.; visualization, M.K.; supervision, M.K. and S.Y.; project administration, M.K.; funding acquisition, M.K. and S.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: We thank Mei Okamoto and Miya Mukai (Kindai University) for providing technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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