Note added after first publication

This version of the ESI replaces the original version published on 27/04/2016, which contained minor errors in the association constants (Tables S1-S3), LOD values (Table S4) and IC_{50} values (Table S5) due to a faulty pH meter. It has since been found that all measured pH values were slightly higher than the true pH values. All experiments performed at a specific pH have been re-run to ensure the main conclusions are unaffected. Please refer to the accompanying Correction article: Chem. Commun., 2016, DOI: 10.1039/c6cc90497a for more information.

Figs S2, S3, S6 and S7, and Tables S1-S5, are herein corrected. The LOD values remain 1000 times lower than the reported experimental conditions adopted by fluorometric methodology.

The Royal Society of Chemistry apologises for these errors and any consequent inconvenience to authors and readers.

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Supporting Information

Triplex-forming peptide nucleic acid modified with 2-aminopyridine as a new tool for detection of A-to-I editing †

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Reagents

All reagents were used as received. RNA oligonucleotides were purchased from Japan Bio Services and were HPLC purified by the manufacturer.

PNA preparation

The PNA synthesis was carried out using solid-phase synthesis methodology and Fmoc chemistry on an Expedite 8909 DNA synthesizer according to previous reports.[1-3] PNAs were purified by RP HPLC and quantified by UV absorption. The structure of PNA was confirmed by MALDI TOF mass spectrometry.

Fluorescence titration assay

Hairpin RNAs labelled with BHQ-1 were prepared in a reaction buffer containing 30 mM HEPES-KOH, 0.01% CHAPS, and 100 mM KCl, at different pHs. Samples were incubated at 95 °C for 5 minutes and cooled to 25 °C at 5 °C min^{-1} to allow proper folding of the hairpin structures. PNA-HF488 (final concentration of 0.05 nM) was mixed with various concentrations of hairpin RNAs (from 0 to 40 nM hairpin RNA) and incubated at 25 °C for 1 hour. The fluorescence signals of PNA-HF488 were measured using a microwell plate reader (Infinite 200 Pro; Tecan) with Corning 384-well, black flat-bottom plates. Excitation and emission wavelength were 490 and 530 nm, respectively. The association constants for the triplex formation at 25 ˚C ($K_{A\ 25}$) were determined by fitting the titration curves obtained from at least three independent sets of experiments. The equation **S1** used is based on a 1:1 binding equilibrium as follows:

$$F = F_{\text{initial}} + \left(\frac{F_{\text{final}} - F_{\text{initial}}}{2 \times [\text{PNA}]} \right) \times \left\{ \left([\text{PNA}] + [\text{RNA}] + \frac{1}{K_{A\,25}} \right) - \sqrt{\left([\text{PNA}] + [\text{RNA}] + \frac{1}{K_{A\,25}} \right)^2 - 4 \times [\text{PNA}] \times [\text{RNA}]} \right\} \quad \textbf{S1}$$

where F is the fluorescence signal of the PNA-HF488 at each concentration of hairpin RNA, F_{initial} is the fluorescence signal of the PNA-HF488 in the absence of hairpin RNA, F_{final} is the fluorescence signal of the PNA-HF488 after binding to hairpin RNA, [PNA]

is the total concentration of the PNA-HF488 (fixed at 0.05 nM), and [RNA] is the hairpin RNA concentration (from 0 to 40 nM).

LOD evaluation

Mixed solutions of the wild-type and the edited hairpin RNAs labelled with BHQ-1 were prepared in different ratios at a total concentration of 100 pM in a reaction buffer containing 30 mM HEPES-KOH (pH 7.0), 0.01% CHAPS, and 100 mM KCl. The solutions were incubated at 90 °C for 5 minutes and cooled to 25 °C at 5 °C min^{-1} to allow proper folding of the hairpin structures. PNA-HF488 (final concentration of 100 pM) was added, and the samples were incubated at 25 °C for 1 hour. Fluorescence signals of PNA-HF488 were recorded as detailed above.

The limit of detection (LOD) for the edited hairpin RNAs was calculated using the following equation:

$$LOD = 3.3 \times \sigma / S \qquad \textbf{S2}$$

where σ is standard deviation of fluorescence intensity of PNA-HF488 in the absence of edited hairpin RNA, and *S* is the slope of the fluorescence vs. concentration of the edited hairpin RNA. Five independent sets of experiments were analyzed.

Competitive binding assay

PNA-HF488 (0.2 nM) and BHQ1-modifiededited hairpin RNAs (0.5nM) were mixed with various concentrations of label-free edited hairpin RNAs in a buffer solution containing 30 mM HEPES-KOH (pH 6.6),100 mM KCl, and 0.01% CHAPS. The mixtures were incubated at 25 ˚C for 1 h, and the fluorescence signals of PNA-HF488 were recorded as detailed above. The half maximal inhibitory concentrations (IC_{50}s) of interaction between the PNA-HF488 and the BHQ1-modified edited hairpin RNA

caused by the label-free edited hairpin RNA were calculated by fitting an Equation S3 as follow:

$$F = F_0 + (F_{final} - F_0) \times \left(\frac{RNA}{IC_{50} + RNA} \right) \qquad \textbf{S3}$$

where F is the fluorescence signal of the PNA-HF488 at each concentration of the label-free edited hairpin RNA, F_0 is the fluorescence signal of the PNA-HF488 in the absence of the label-free edited hairpin RNA, F_{final} is the fluorescence signal at saturation, RNA is the concentration of the label-free edited hairpin RNA.

References

1. T. Zengeya, P. Gupta and E. Rozners, *Angew. Chem. Int. Ed*., 2012, **51**, 12593.
2. P. Gupta, T. Zengeya and E. Rozners, *Chem. Commun*., 2011, **47**, 11125.
3. T. Endoh, D. Hnedzko, E. Rozners and N. Sugimoto, *Angew. Chem. Int. Ed.*, 2016, **55**, 899.

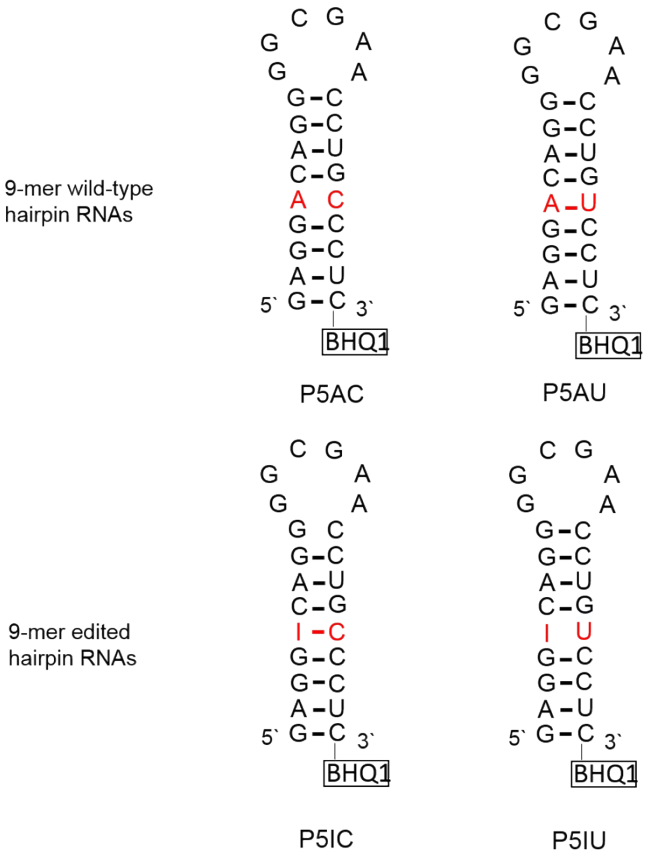

Fig. S1 Predicted secondary structures of hairpin RNAs.

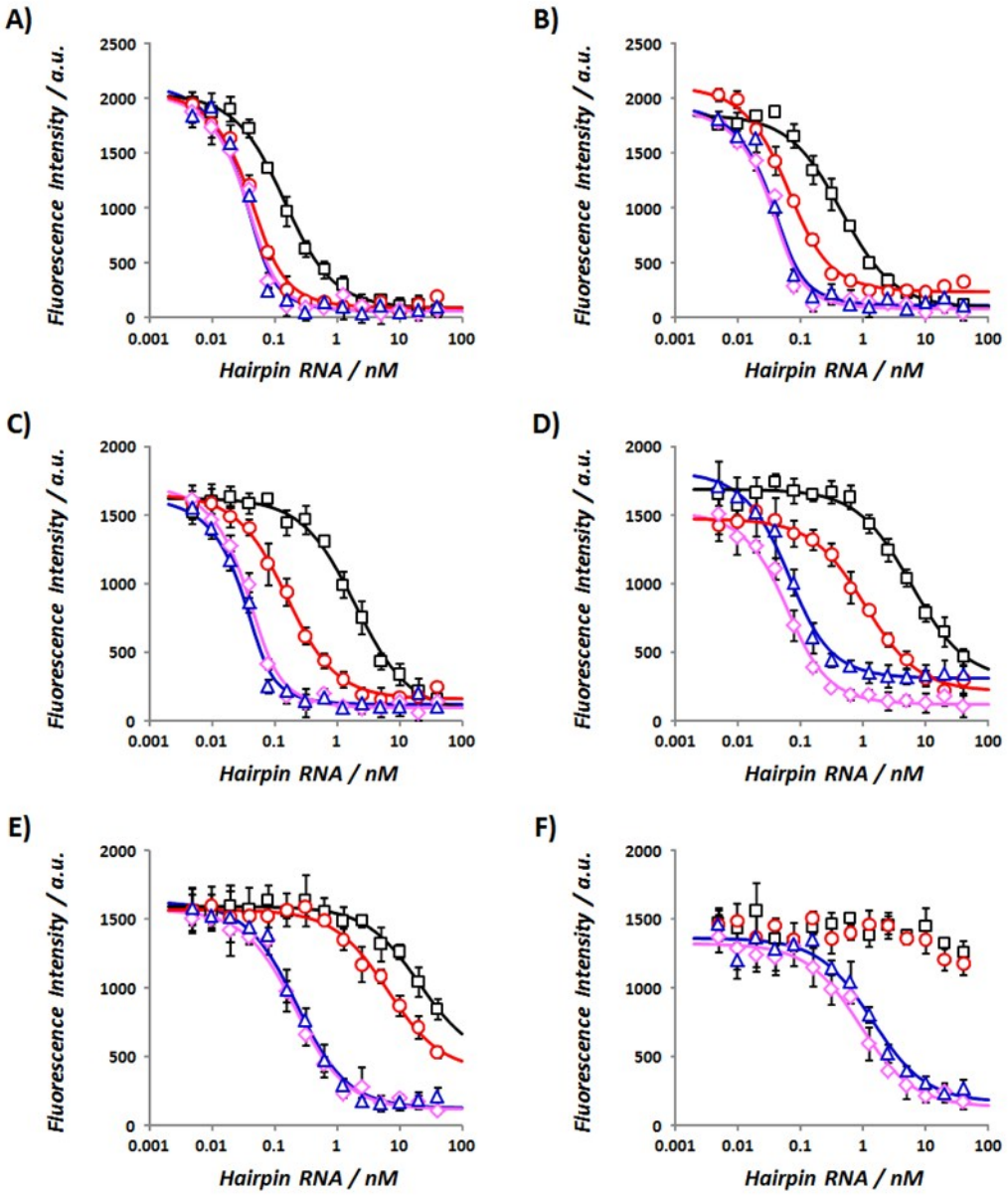

Fig. S2 Fluorescence intensities of PNA-HF488 mixed with various concentrations of P5AC (black), P5AU (red), P5IC (blue), and P5IU (pink) in a buffer containing 30 mM HEPES-KOH, 100 mM KCl, and 0.01% CHAPS at 25 ˚C at A) pH 6.4, B) pH 6.6, C) pH 6.8, D) pH 7.0, E) pH 7.2, and F) pH 7.4.

Table S1 Relative K_{A25} values for interaction between PNA-HF488 and hairpin RNAs under different pH conditions at 25 ˚C

Hairpin RNA	Relative K_{A25} (edited/wild-type)				
	pH 6.4	pH 6.6	pH 6.8	pH 7.0	pH 7.2
P5AC P5IC	14	40	269	157	102
P5AU P5IU	1.6	3.9	11	30	34

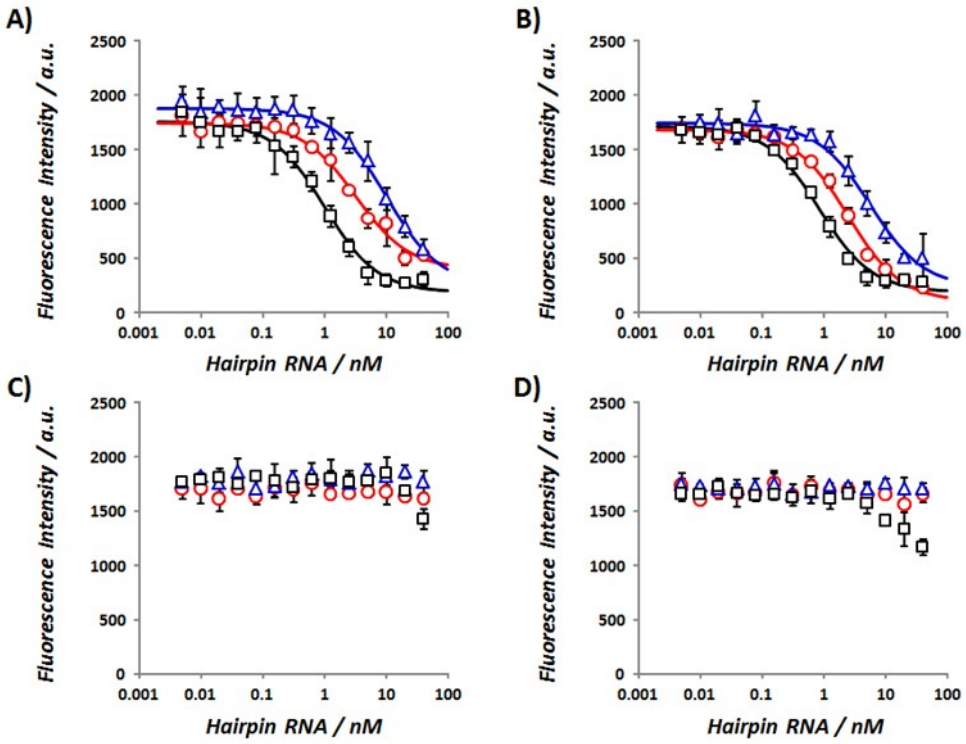

Fig. S3 Fluorescence intensities of PNA-HF488 mixed with various concentrations of (A) P5IC, (B) P5IU, (C) P5AC, and (D) P5AU in a buffer containing 30 mM HEPES-KOH, pH 7.0, and 0.01% CHAPS at 25 ˚C in the presence of 200 mM KCl (black), 250 mM KCl (red), and 300 mM KCl (blue).

Table S2 K_{A25} values for interaction between PNA-HF488 and hairpin RNAs under different salt concentrations at pH 7.0 and 25 ˚C[a]

Hairpin RNA	$K_{A\ 25}$ ($\times 10^8\ M^{-1}$)		
	200 mM KCl	250 mM KCl	300 mM KCl
P5AC	n.d.[b]	n.d.[b]	n.d.[b]
P5IC	10.3 ± 1.3	3.15 ± 0.36	0.979 ± 0.10
P5AU	n.d.[b]	n.d.[b]	n.d.[b]
P5IU	12.2 ± 1.3	4.11 ± 0.06	1.77 ± 0.02

a) The reported values are means ± standard deviations from at least three independent measurements.

b) The value could not be determined due to low affinity.

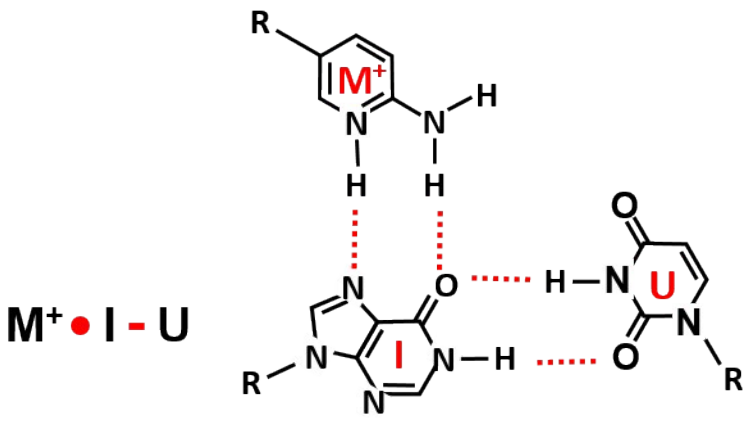

Fig. S4 Schematic representation of the M^+•I-U base triad involving the I-U wobble base pair.

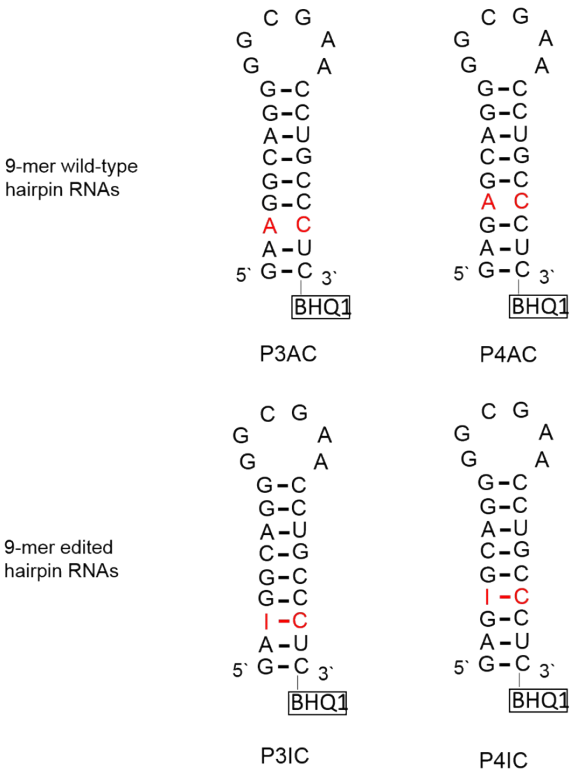

Fig. S5 Predicted secondary structures of the additional hairpin RNAs.

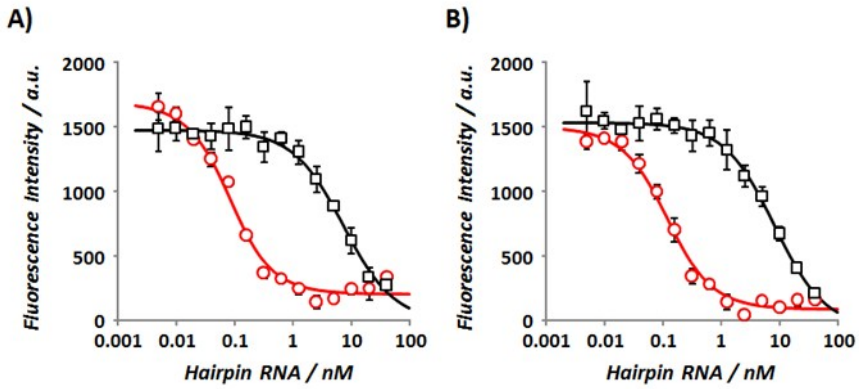

Fig. S6 Fluorescence intensities of PNA-HF488 mixed with various concentrations of (A) P3AC (black) and P3IC (red), and (B) P4AC (black) and P4IC (red), in a buffer containing 30 mM HEPES-KOH, pH 7.0, 100 mM KCl, and 0.01% CHAPS at 25 ˚C.

Table S3 K_{A25} values between PNA-HF488 and hairpin RNAs obtained by fluorescence titration at pH 7.0 and 25 °C[(a)]

Hairpin RNA	K_{A25} ($\times 10^9$ M^{-1})	Relative K_{A25} (edited/wild-type)
P3AC	0.136 ± 0.02	129
P3IC	17.6 ± 0.71	
P4AC	0.122 ± 0.01	89
P4IC	10.8 ± 0.94	

a) The reported values correspond to mean ± standard deviation from at least three independent measurements.

Table S4 LOD values calculated from Equation S2 for the edited hairpin RNAs[(a)]

Hairpin RNA	LOD (pM)
P3IC-BHQ1	17.7 ± 1.3
P4IC-BHQ1	23.7 ± 1.9
P5IC-BHQ1	13.0 ± 0.7
P5IU-BHQ1	17.3 ± 2.3

a) The values are means ± standard deviations from at least five independent measurements.

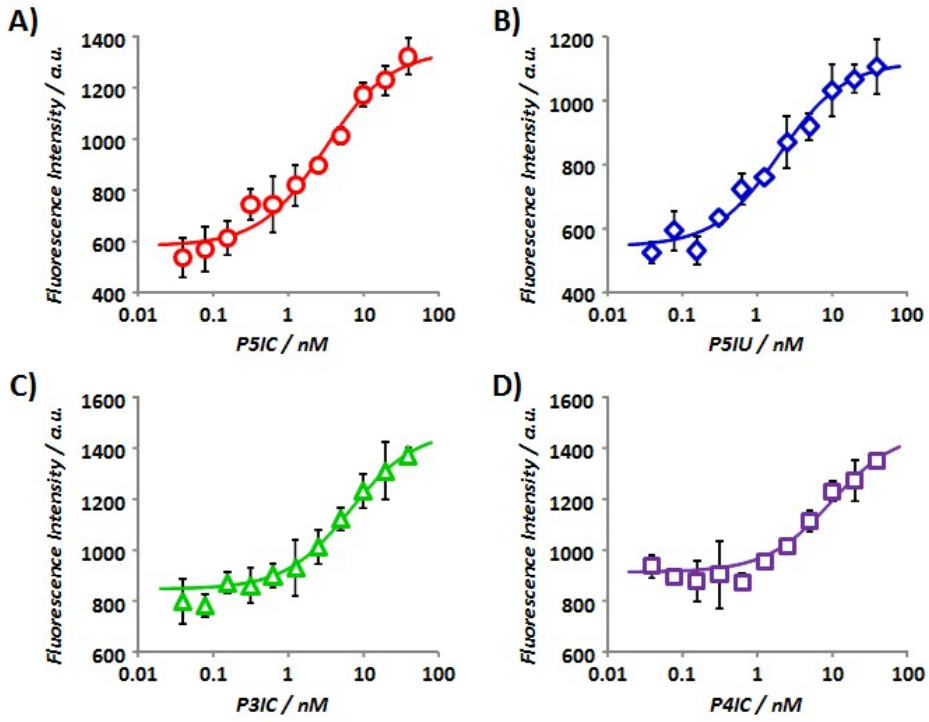

Fig. S7 PNA-HF488 (0.2 nM) was mixed with BHQ1-modified edited hairpin RNA (0.5 nM) and indicated concentrations of the cognate label-free edited hairpin RNA, A) P5IC, B) P5IU, C) P3IC, or D) P4IC, in a buffer solution containing 30 mM HEPES-KOH (pH 7.0), 100 mM KCl, and 0.01% CHAPS. Fluorescence signals of the PNA-HF488 were evaluated at 25 °C after 1 h using 490 nm excitation and 530 nm emission. Values are means ± standard deviations of triplicated samples.

Table S5 IC_{50} values calculated from Equation S3 for the edited hairpin RNAs[a,b]

Hairpin RNA	IC_{50} (nM)
P3IC (label-free)	7.0 ± 2.1
P4IC (label-free)	9.7 ± 0.7
P5IC (label-free)	3.1 ± 0.3
P5IU (label-free)	2.4 ± 1.2

a) The values are means ± standard deviations from at least three independent measurements.

b) Fluorescence measurements were performed in buffer at pH 6.6.