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Supplementary Information

Peptide Nucleic Acid-Templated Selenocystine-Selenoester Ligation Enables Rapid miRNA Detection

Jessica Sayers,[1,2] *Richard J. Payne*[1*] *and Nicolas Winssinger*[2*]

[1] School of Chemistry, The University of Sydney, Building F11, Sydney, Australia

Fax: +61 2 9351 3329; Tel: +61 2 9351 5877

E-mail: richard.payne@sydney.edu.au

[2] Department of Organic Chemistry, University of Geneva, Quai Ernest Ansermet 30, 1211 Geneva 4, Switzerland

Fax: +41 22 379 32 15; Tel: +41 22 379 61 05

E-mail: nicolas.winssinger@unige.ch

Contents

General

UPLC-MS spectra were recorded using a DIONEX Ultimate 3000 UHPLC with a Thermo LCQ Fleet Mass Spectrometer System using a PINNACLE DB C18 column (1.9 mm, 50 x 2.1 mm) with electrospray ionization in positive mode (ESI). A linear elution gradient from 95% H_2O (0.5% HCO_2H) to 100% MeCN at a flow rate of 0.5 mL/min was used unless otherwise stated.

HPLC purification was performed by Agilent Technologies 1260 infinity using a ZORBAX 300SB-C18 column (9.4 x 250 mm) at a flow rate of 0.2 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B), unless otherwise noted.

MALDI-TOF Mass spectra were measured using a Bruker Daltonics Autoflex TOF/TOF spectrometer. The samples were prepared using 2,5-dihydroxybenzoic acid (DHB) as a matrix.

Automated solid phase synthesis was carried out on an Intavis Multipep instrument (http://www.intavis.com/en/Automated_Peptide_Synthesis/MultiPep_RS/index.php).

Materials

Commercial materials were used as received unless otherwise noted. Amino acids, coupling reagents and resins were obtained from Novabiochem or GL Biochem. Reagents that were not commercially available were synthesized following literature procedures and referenced accordingly. N,N-dimethylformamide (DMF) was obtained as peptide synthesis grade from Merck or Labscan.

PNA monomers with Boc-protected nucleobases were prepared according to previously reported procedures (S. Pothukanuri, Z. Pianowski, N. Winssinger, Eur. J. Org. Chem., 2008, 18, 3141-48).

Solid-Phase Synthesis

Preloading NovaPEG Rink Amide Resin

Rink amide resin (0.53 mmol/g loading) was initially washed with CH_2Cl_2 (5 x 3 mL) and DMF (5 x 3 mL). HATU (2.8 eq.), N,N-diisopropylethylamine (DIPEA) (5.0 eq.) and 2,6-lutidine (3.0 eq.) were added to a solution of Fmoc-XX-OH (3.0 eq., where XX = a PNA monomer or an amino acid) in NMP (final concentration 0.1 M). After 2 min of pre-activation, the mixture was added to the resin. After 2 h the resin was washed with DMF (5 x 3 mL), CH_2Cl_2 (5 x 3 mL) and DMF (5 x 3 mL), capped with a solution of Ac_2O (5.3 equiv) and 2,6-lutidine (6.4 equiv) in DMF (3 mL, 2 x 3 min) and washed with DMF (5 x 3 mL), CH_2Cl_2 (5 x 3 mL) and DMF (5 x 3 mL). The resin was subsequently submitted to iterative solid-phase synthesis.

Preloading 2-chloro-trityl chloride resin

2-chlorotrityl chloride resin (1.60 mmol/g loading) was swollen in dry CH_2Cl_2 for 30 min then washed with CH_2Cl_2 (5 x 3 mL) and DMF (5 x 3 mL). A solution of a suitably protected Fmoc-XX-OH (3.0 eq.), DIPEA (5.0 eq.) and 2,6-lutidine (3.0 eq.) in NMP (final concentration 0.1 M) was added and the resin shaken at room temperature for 2 h. The resin was washed with DMF (5 x 3 mL) and CH_2Cl_2 (5 x 3 mL) and treated with a solution of CH_2Cl_2/CH_3OH/DIPEA (17:2:1 v/v/v, 3 mL) for 30 min. The resin was washed with CH_2Cl_2 (5 x 3 mL), and DMF (5 x 3 mL) and subsequently submitted to iterative solid-phase synthesis.

General Iterative PNA/Peptide Assembly (Fmoc/Mtt-SPPS)

1) ***Either:***

   Fmoc Deprotection: The resin was treated with 20% piperidine/DMF (3 mL, 2 × 3 min) and washed with DMF (5 × 3 mL), CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL).

   Or:

   Mtt Deprotection: The resin was treated with a 1:1 solution of HFIP in DCE containing 80 mM HOBt (2 x 3 min) and washed with CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL).

2) *General Coupling:* A solution of protected PNA monomer or amino acid (3.0 eq.), HATU (2.8 eq.) and DIPEA (5.0 eq.) in NMP (final concentration 0.1 M) was added to the resin. After 2 h, the resin was washed with DMF (5 × 3 mL), CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL).

3) *Capping:* A solution of Ac_2O (5.3 equiv) and 2,6-lutidine (6.4 equiv) in DMF was added to the resin (3 mL). After 3 min the resin was washed with DMF (5 × 3 mL), CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL).

Cleavage: An acidic cocktail comprising TFA/*i*Pr_3SiH/H_2O (95:2.5:2.5, v/v/v, 3 mL) was added to the resin. After 1 h the resin was washed with TFA (3 × 2 mL).

Work-up: The combined solutions were concentrated under a stream of nitrogen. The residue was dissolved in H_2O containing 0.1% TFA, filtered and purified by preparative HPLC and analyzed by LC-MS and ESI mass spectrometry.

Automated PNA Synthesis

PNA synthesis was performed using an automated peptide synthesizer (Intavis MultiPep instrument in 500 µL fritted tubes.). The resin (5 mg, 1 µmol per column) was swollen in DCM (300 µL) for 20 min. Standard couplings were performed by treatment of the resin with a preactivated (5 min) solution of the corresponding protected PNA monomer/amino acid (5 eq.), HATU (4 eq.), DIPEA (5 eq.), and 2,6-lutidine (7.5 eq.) for 20 min in NMP. This process was repeated once (double couplings). Each coupling was followed by a capping step with Ac_2O (5.3 eq.) and 2,6-lutidine (6.4 eq.) in DMF (150 µL per column).

General Procedures

Solution Phase Selenoester Synthesis

Cleavage of the PNA-peptides from the resin was effected by treating with 30 vol.% HFIP in DCM for 2 h before concentrating *in vacuo*. The resulting residue was dissolved in anhydrous DMF and cooled to 0 °C. Diphenyl diselenide (DPDS) (2.5 eq. in DMF) was added to the solution followed by Bu_3P (5 eq.). The reaction was allowed to proceed at 0 °C for 3 h, after which time the solvent was removed *in vacuo*. The crude material was put on ice and the protecting groups removed via treatment with TFA:TIS:H_2O (95:2.5:2.5 v/v/v). After 1 h at room temperature the cleavage cocktail was removed under a stream of N_2 and the crude residue suspended in diethyl ether and cooled to -20 °C. The precipitate was pelleted by centrifugation at 4000 rpm for 5 min, the supernatant decanted and the pellet dissolved in 0.1% TFA/H_2O with addition of 0.1% TFA/MeCN and purified via preparative HPLC.

Solid Phase Selenoester Synthesis

Following elongation of the PNA/peptide, the resin was swollen in anhydrous CH_2Cl_2 for 5 mins. The vessel was then purged with N_2, the solvent drained and a solution of $Pd(PPh_3)_4$ (0.25 eq.) in

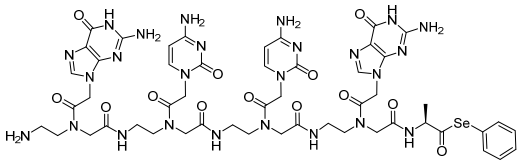
anhydrous CH_2Cl_2 was added, followed by $PhSiH_3$ (24 eq.) maintaining an inert atmosphere. The resin was then gently agitated for 1 h. The resin was then drained and washed with CH_2Cl_2 (5 x 3 mL) and DMF (5 x 3 mL). To effect the selenoesterification reaction, a solution of DPDS (2.5 eq.) and Bu_3P (5.0 eq.) in anhydrous DMF was added to the resin in an inert atmosphere. The resin was agitated for 3 h and then washed with DMF (5 x 3 mL), CH_2Cl_2 (5 x 3 mL) and DMF (5 x 3 mL). The crude material was put on ice and the protecting groups removed via treatment with TFA:TIS:H_2O (95:2.5:2.5 v/v/v). After 1 h at room temperature the cleavage cocktail was removed under a stream of N_2 and the crude residue suspended in diethyl ether and cooled to -20 °C. The precipitate was pelleted by centrifugation at 4000 rpm for 5 min, the supernatant decanted and the pellet dissolved in 0.1% TFA/H_2O with addition of 0.1% TFA/MeCN and purified via preparative HPLC.

Selenocystine-selenoester Ligation Reaction

PNA-peptide selenoesters and PNA-peptide dimers bearing N-terminal selenocystine were dissolved in DMF as 1 mM stock solutions. Aliquots of the selenoester (1.0 eq.) and diselenide dimer (0.5 eq.) stock solutions were taken and added to PBS buffer to the desired overall concentration. The ligation reaction was gently agitated and allowed to proceed for the specified time before analysis by LCMS.

Synthesis of Model PNA-Peptides

PNA-Peptide Selenoester (1) (H_2N-[GCCG]-Ala-SePh)



2-chlorotrityl chloride resin (50 mg, 1.6 mmol/g, 80 µmol) was loaded with Fmoc-Ala-OH (75 mg, 3 eq., 240 µmol) as outlined in the general pre-loading procedure, and extended at the N-terminus with PNA monomers via automated Fmoc-strategy solid-phase synthesis to the desired sequence length. The protected PNA-peptide was cleaved from the resin using 30 vol.% HFIP in DCM and selenoesterified as outlined in the general methods section. The crude residue was purified by preparative reverse-phase HPLC (10 to 50% B over 15 min, 0.1% TFA) and lyophilized to afford the desired selenoester **1** as a white solid following lyophilisation. Formula: $C_{51}H_{63}N_{25}O_{13}Se$; Calculated M_W 1313.19; Yield: 8.6 mg, 6.6 µmol, 8%.

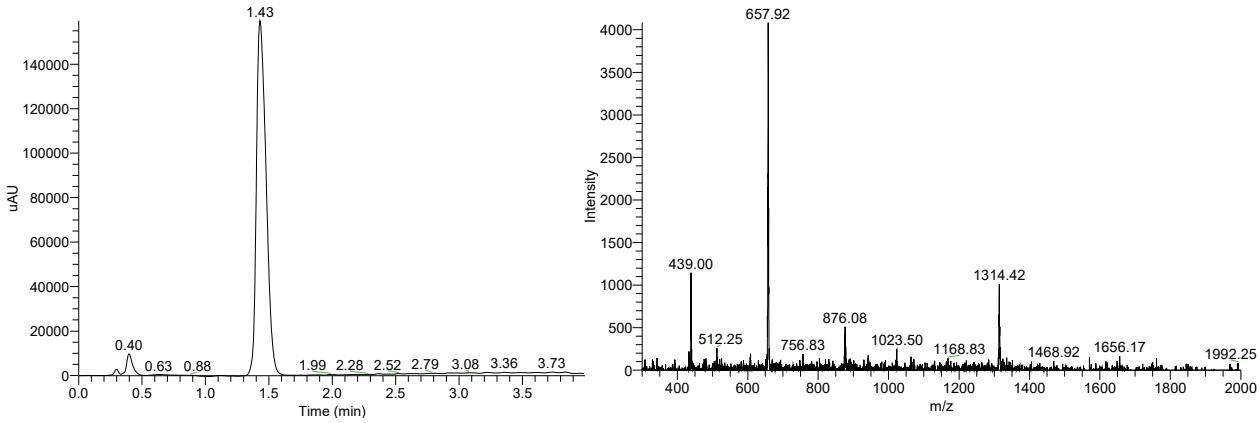


Figure S1. Analytical UPLC trace of HPLC purified **1**; R_t 1.43 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+H]^+$ 1314.19, $[M+2H]^{2+}$ 657.60, $[M+3H]^{3+}$ 438.73; Mass Found (ESI+): $[M+H]^+$ 1314.42, $[M+2H]^{2+}$ 657.92, $[M+3H]^{3+}$ 439.00.

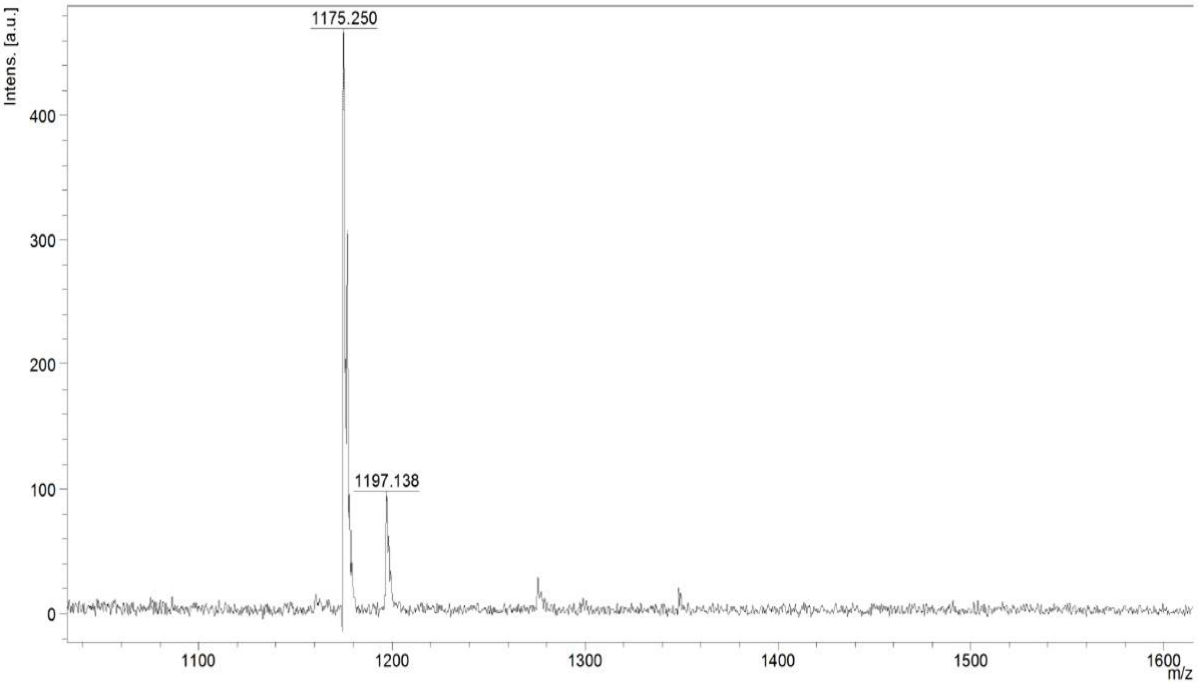
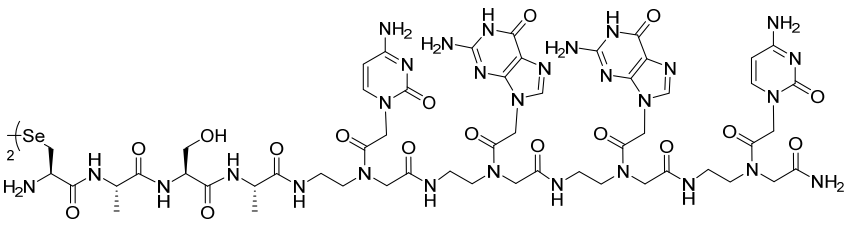


Figure S2. MALDI-TOF spectrum of HPLC purified **1**; Calculated *m/z* 1174.19, found 1175.25 $[M+H_2O\text{-}SePh]^+$ (hydrolysis occurring during MALDI preparation) and 1197.138 $[M+NaOH\text{-}SePh]^+$

PNA-Peptide Diselenide Dimer (2) (H_2N-SecAlaSerAla-[CGGC]-$CONH_2$)$_2$



NovaPEG Rink amide resin (20 mg, 0.5 mmol/g, 10 µmol) was swollen in CH_2Cl_2 for 30 minutes. The desired PNA-peptide sequence was then elongated *via* automated Fmoc-SPPS as outlined in the general procedures. Coupling of (Boc-Sec-OH)$_2$ was then performed manually by treatment of the resin with a pre-activated solution of (Boc-Sec-OH)$_2$ (5.3 mg, 1.0 eq., 10 µmol), HOAt (33 µL, 2 eq., 20 µmol) and DIC (3.1 µL, 2 eq., 20 µmol) in NMP (final concentration 0.1 M). After 6 h of agitation at room temperature, the resin was washed with DMF (5 x 3 mL), CH_2Cl_2 (5 x 3mL) and DMF (5 x 3 mL). The fully extended PNA-peptide was cleaved from the solid support by treatment with an acidic cocktail of TFA/*i*Pr_3SiH/H_2O (95:2.5:2.5, v/v/v, 3 mL) for 1 h and then washed with TFA (2 x 2 mL). Crude **2** was precipitated from cold Et_2O and purified by reverse-phase semi-preparative HPLC (10-50% B over 12 min, 0.1 % TFA) to yield **2** exclusively as the diselenide dimer following lyophilsation. Formula: $C_{54}H_{74}N_{29}O_{17}Se$; Calculated Diselenide M_W 2962.00; Yield: 2.5 mg, 1.7 µmol, 17%.

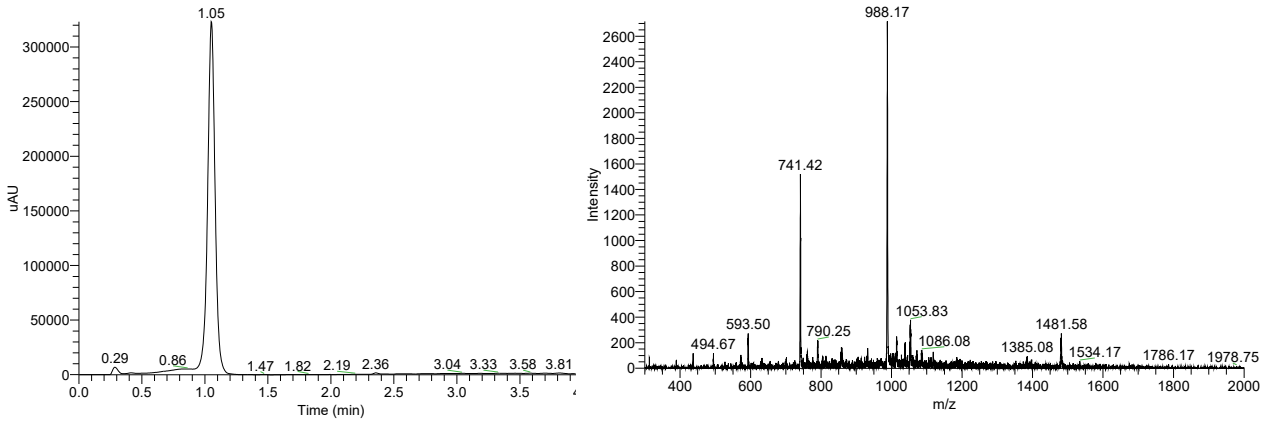


Figure S3. Analytical UPLC trace of HPLC purified **2**; R_t 1.05 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+2H]^{2+}$: 1482.00, $[M+3H]^{3+}$ 988.33; Mass Found (ESI+): $[M+2H]^{2+}$ 1481.58, $[M+3H]^{3+}$ 988.17, $[M+4H]^{4+}$ 741.42.

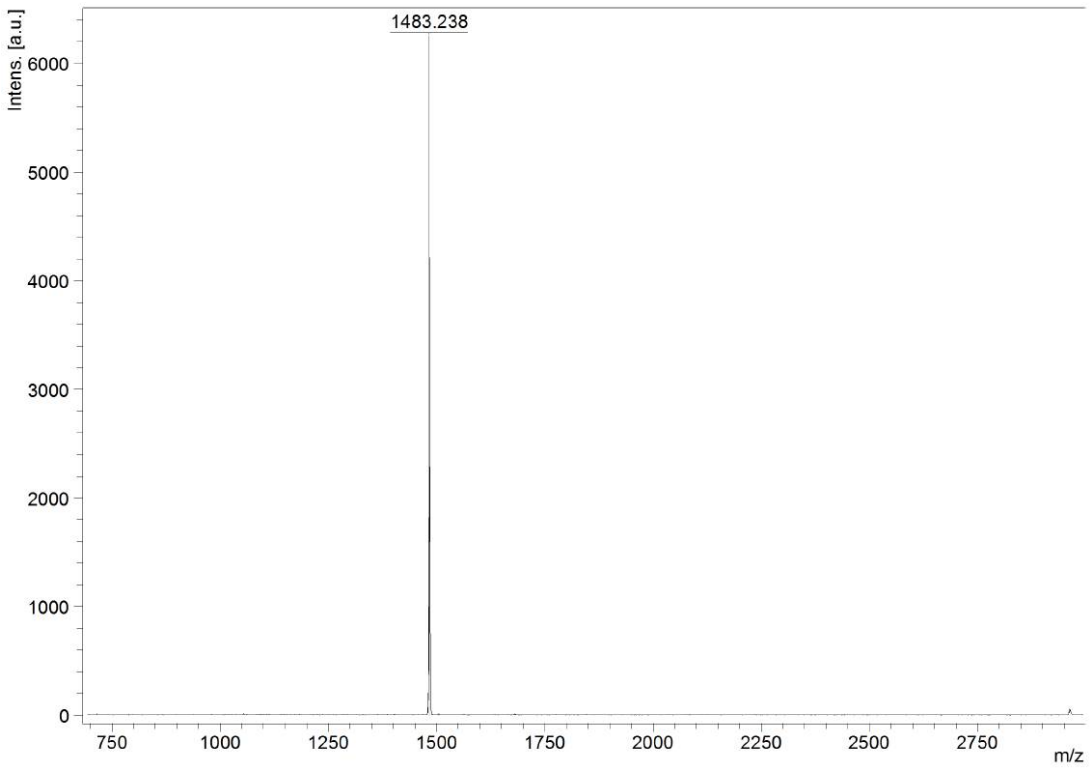
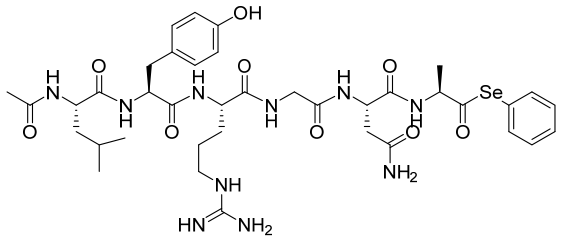


Figure S4. MALDI-TOF spectrum of HPLC purified **2**; Calculated Selenol *m/z* 1483.00, found 1483.24 $[M+H]^+$.

Peptide Selenoester (6) (Ac-LYRGNA-SePh)



Peptide selenoester **6** (80 µmol scale) was prepared as outlined in the general procedures and isolated as a white solid following purification by preparative HPLC (10 to 50% B over 12 min, 0.1% TFA) and lyophilisation. Formula: $C_{38}H_{54}N_{10}O_9Se$; Calculated M_W 873.87; Yield: 17.3 mg, 19.7 µmol, 25%.

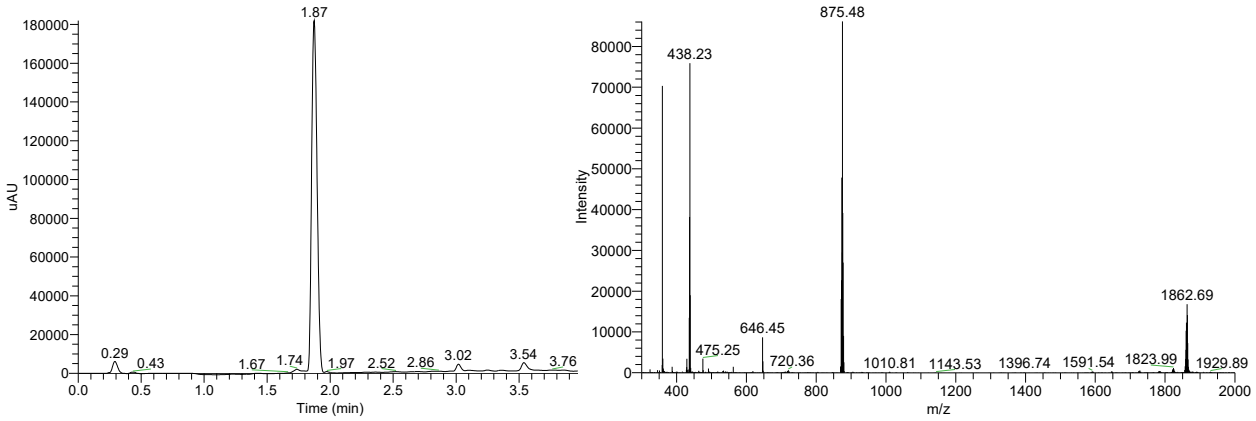


Figure S5. Analytical UPLC trace of HPLC purified **6**; R_t 1.87 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+H]^+$ 874.87, $[M+2H]^{2+}$ 437.94;Mass Found (ESI+): $[M+H]^+$ 875.48 , $[M+2H]^{2+}$ 438.23.

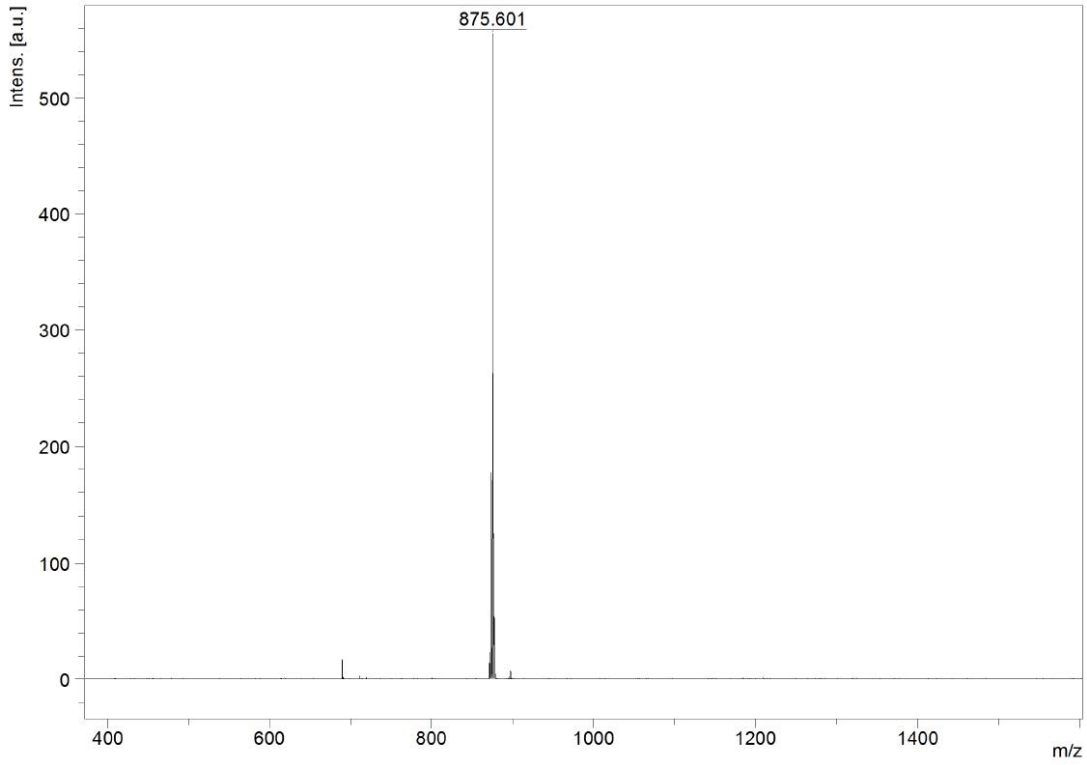
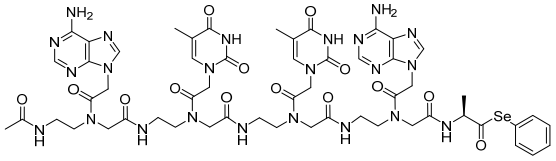


Figure S6. MALDI-TOF spectrum of HPLC purified **6**; Calculated *m/z* 874.87, found 875.601 $[M+H]^+$.

PNA-Peptide Selenoester (7) (H_2N-[ATTA]-Ala-SePh)



2-chlorotrityl chloride resin (50 mg, 1.6 mmol/g, 80 µmol) was loaded with Fmoc-Ala-OH (75 mg, 3 eq., 240 µmol) as outlined in the general coupling procedure, and extended at the N-terminus with PNA monomers via automated Fmoc-SPPS to the desired sequence length. The protected PNA-peptide was cleaved from the resin using 30 vol.% HFIP in DCM and selenoesterified as outlined in the general methods section. The crude residue was purified by preparative reverse-phase HPLC (10 to 50% B over 15 min, 0.1% TFA) and lyophilized to afford the desired selenoester **7** as a white solid following lyophilisation. Formula: $C_{55}H_{67}N_{23}O_{14}Se$; Calculated M_W 1353.44; Yield: 6.9 mg, 5.1 µmol, 6%.

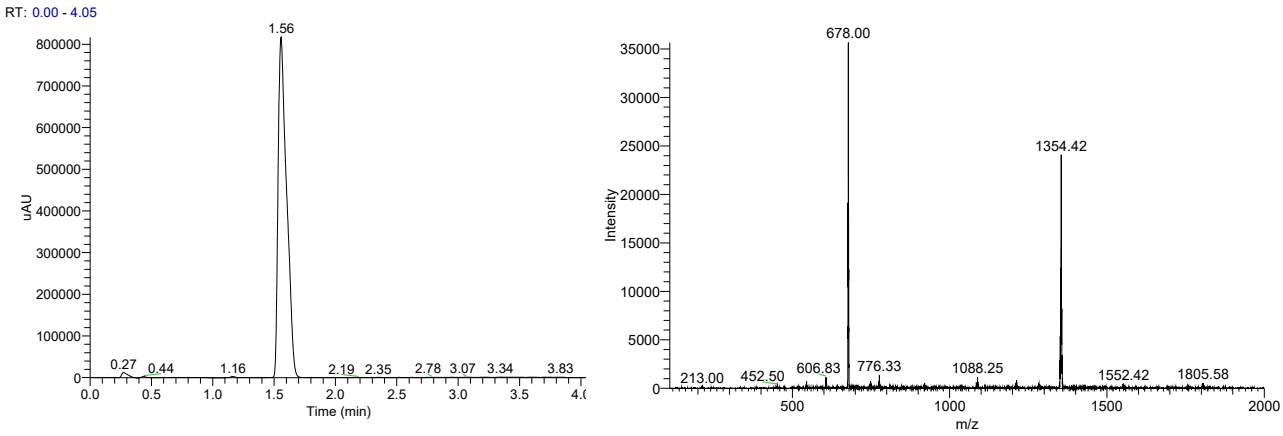


Figure S7. Analytical UPLC trace of HPLC purified **7**; R_t 1.56 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+H]^+$ 1354.44, $[M+2H]^{2+}$ 678.22; Mass Found (ESI+): $[M+H]^+$ 1354.42, $[M+2H]^{2+}$ 678.00.

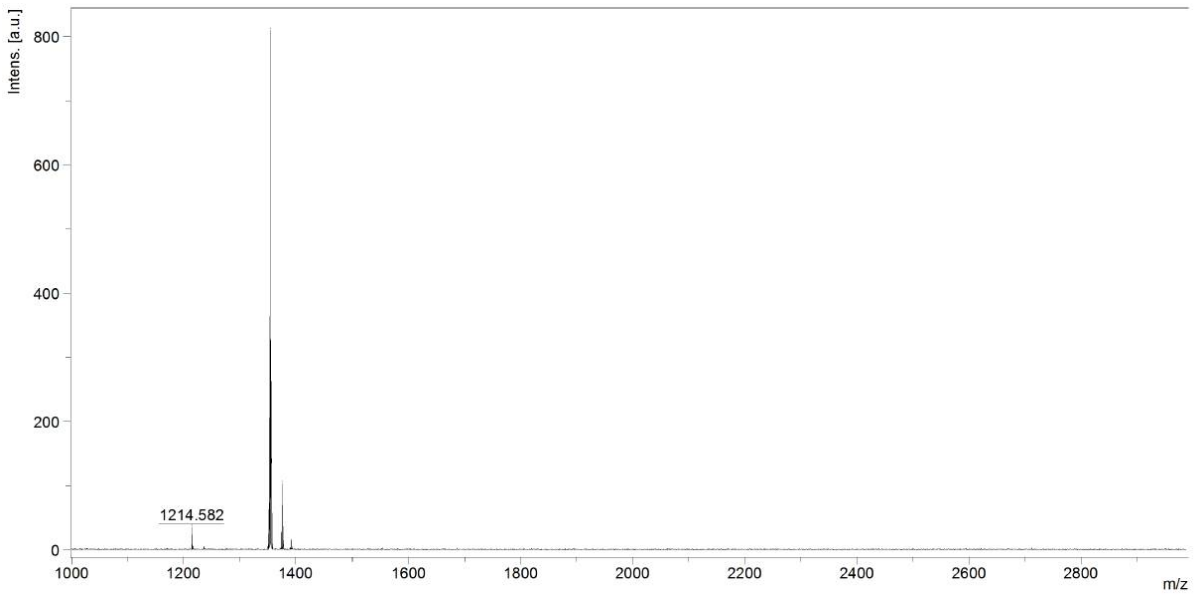
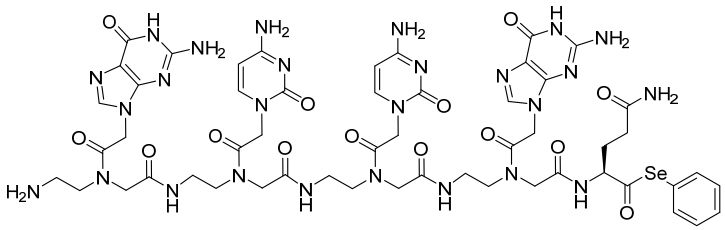


Figure S8. MALDI-TOF spectrum of HPLC purified **7**; Calculated *m/z* 1354.44, found 1354.596 $[M+H]^+$ and $[M+H_2O\text{-SePh}]^+$ 1214.582.

PNA-Peptide Selenoester (8) (H_2N-[GCCG]-Gln-SePh)



NovaPEG Rink amide resin (30 mg, 0.5 mmol/g, 15 µmol) was loaded with Fmoc-Glu-OAll (6.1 mg, 1 eq., 15 µmol) as outlined in the general coupling procedure, and extended at the N-terminus with PNA monomers via automated Fmoc-SPPS to the desired sequence length. The resin was then solvated in anhydrous CH_2Cl_2 for 5 minutes before $PhSiH_3$ (44 µL, 24 eq., 360 µmol) and $Pd(PPh_3)_4$ (4.3 mg, 0.25 eq., 3.75 µmol) were added in CH_2Cl_2, maintaining an inert atmosphere. The vessel was gently agitated at room temperature before washing with CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL). The resulting deprotected C-terminus was then selenoesterified on-resin as outlined in the general procedures. Cleavage from the solid support was effected by treatment with an acidic cocktail (TFA/TIS/H_2O 95:2.5:2.5) for 1 h and then concentrated under a stream of N_2. Following precipitation from Et_2O and purification by preparative HPLC (10 to 50% B over 15 min, 0.1% TFA), the desired selenoester **8** was obtained as a white solid after lyophilisation. Formula: $C_{53}H_{66}N_{26}O_{14}Se$; Calculated M_W 1370.24; Yield: 1.9 mg, 1.4 µmol, 9%.

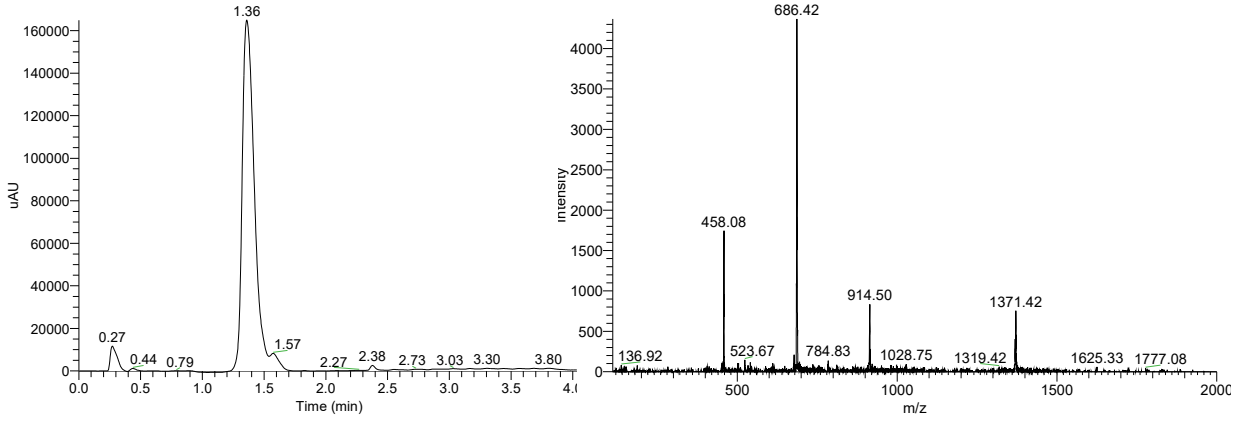


Figure S9. Analytical UPLC trace of HPLC purified **8**; R_t 1.36 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+H]^+$ 1371.24, $[M+2H]^{2+}$ 686.12, $[M+3H]^{3+}$ 457.75; Mass Found (ESI+): $[M+H]^+$ 1371.42, $[M+2H]^{2+}$ 686.42, $[M+3H]^{3+}$ 458.08.

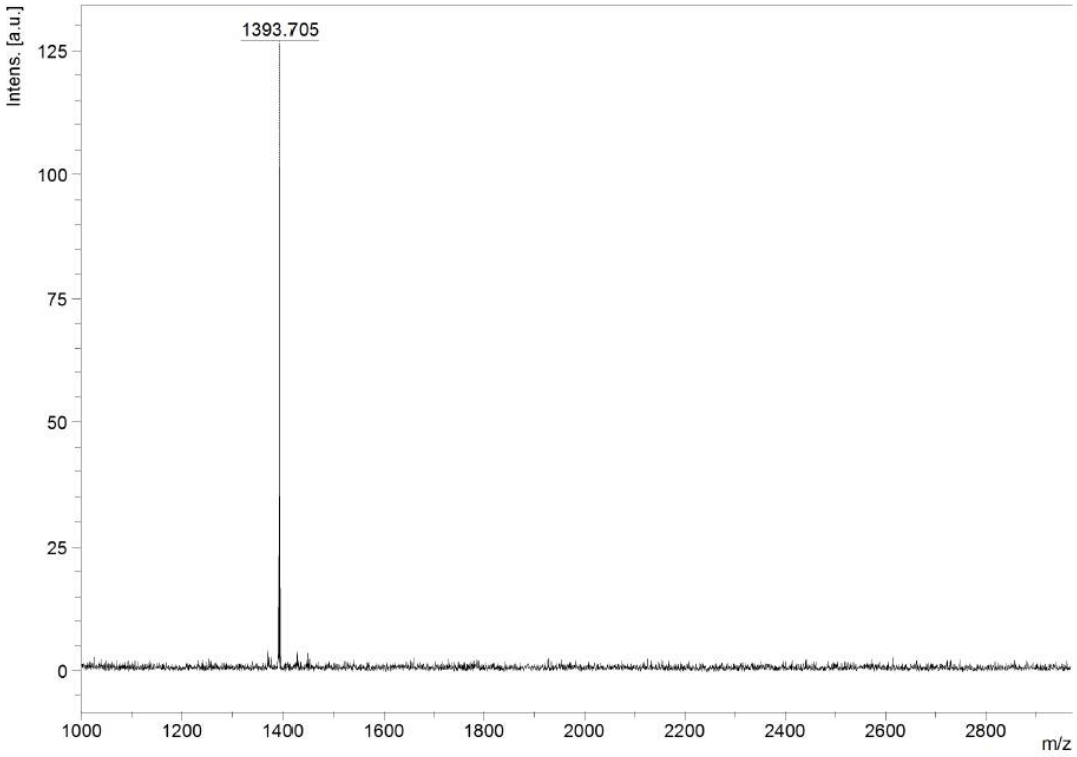
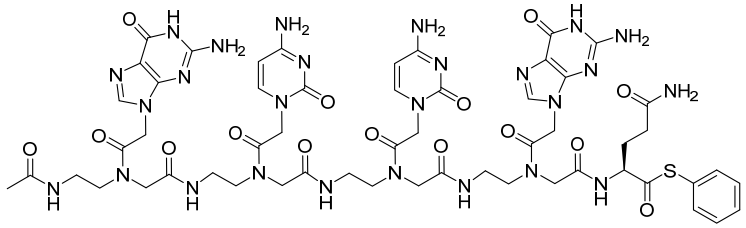


Figure S10. MALDI-TOF spectrum of HPLC purified **8**; Calculated *m/z* 1393.24, found 1393.705 $[M+Na]^+$.

PNA-Peptide Thioester (9) (H_2N-[GCCG]-Gln-SPh)



NovaPEG Rink amide resin (10 mg, 0.5 mmol/g, 5 µmol) was loaded with Fmoc-Glu-OAll (3.0 mg, 1 eq., 5 µmol) as outlined in the general coupling procedure, and extended at the N-terminus with PNA monomers via automated Fmoc-SPPS to the desired sequence length. The resin was then solvated in anhydrous CH_2Cl_2 for 5 minutes before $PhSiH_3$ (15 µL, 24 eq., 120 µmol) and $Pd(PPh_3)_4$ (1.4 mg, 0.25 eq., 1.25 µmol) were added in CH_2Cl_2, maintaining an inert atmosphere. The vessel was gently agitated at room temperature before washing with CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL). The resulting deprotected C-terminus was then thioesterified on-resin as outlined in the general procedures using diphenyl disulfide in place of diphenyl diselenide. Cleavage from the solid support was effected by treatment with an acidic cocktail (TFA/TIS/H_2O 95:2.5:2.5) for 1 h and then concentrated under a stream of N_2. Following precipitation from Et_2O and purification by preparative HPLC (10 to 50% B over 15 min, 0.1% TFA), the desired thioester **9** was obtained as a white solid after lyophilisation. Formula: $C_{53}H_{66}N_{26}O_{14}S$; Calculated M_W 1365.38; Yield: 0.51 mg, 0.37 µmol, 7.5%.

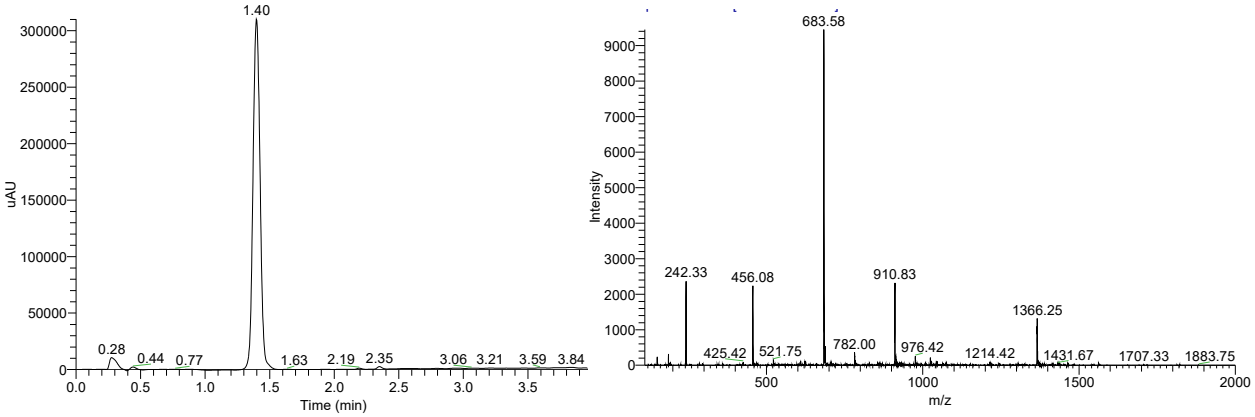


Figure S11. Analytical UPLC trace of HPLC purified **9**; R_t 1.40 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+H]^+$ 1366.38, $[M+2H]^{2+}$ 683.69, $[M+3H]^{3+}$ 456.13; Mass Found (ESI+): $[M+H]^+$ 1366.25, $[M+2H]^{2+}$ 683.58, $[M+3H]^{3+}$ 456.08.

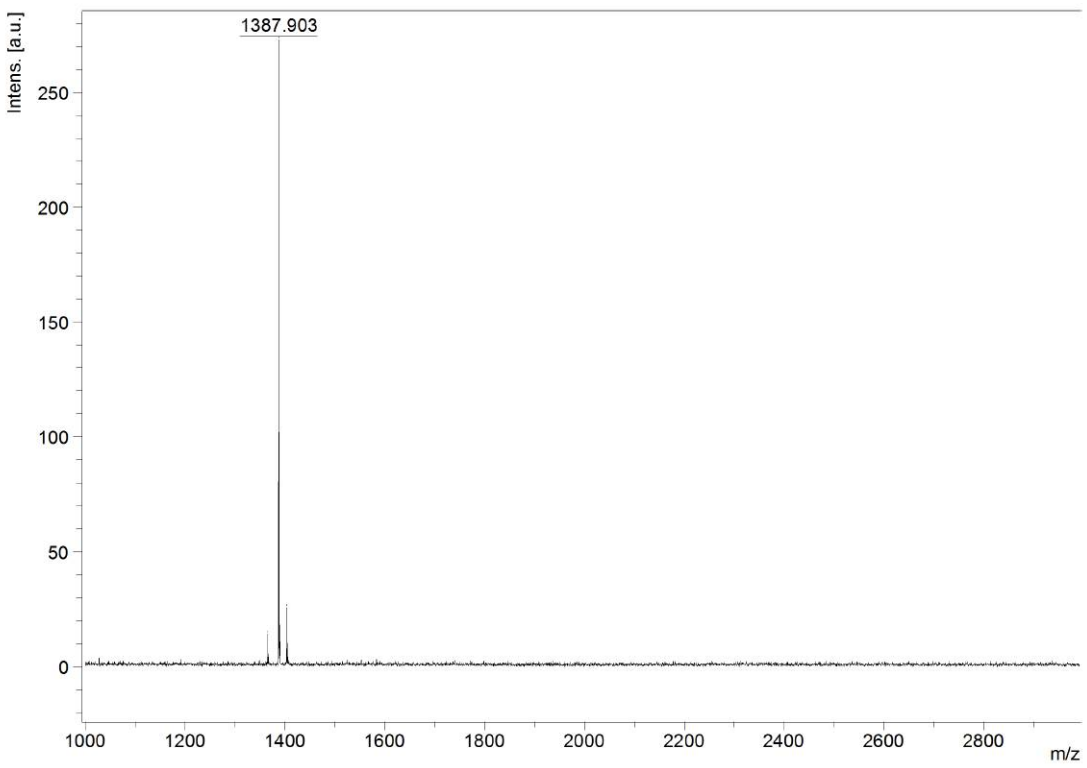
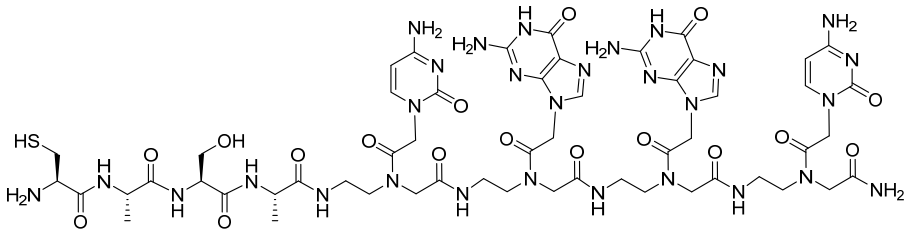


Figure S12. MALDI-TOF spectrum of HPLC purified **9**; Calculated *m/z* 1388.37 $[M+Na]^+$, found 1387.903 $[M+Na]^+$.

PNA-Peptide (10) (H_2N-CysAlaSerAla-[CGGC]-$CONH_2$)



NovaPEG Rink amide resin (30 mg, 0.5 mmol/g, 15 µmol) was swollen in CH_2Cl_2 for 30 minutes. The desired PNA-peptide sequence was then elongated *via* automated Fmoc-SPPS as outlined in the general procedures. The fully extended PNA-peptide was cleaved from the solid support by treatment with an acidic cocktail of TFA/iPr$_3$SiH/H_2O (95:2.5:2.5, v/v/v, 3 mL) for 1 h and then washed with TFA (2 x 2 mL). Crude **10** was precipitated from cold Et_2O and purified by reverse-phase semi-preparative HPLC (10-50% B over 12 min, 0.1 % TFA) to yield **10** as a white solid following lyophilisation. Formula: $C_{54}H_{75}N_{29}O_{17}S$. Calculated M_W 1434.44; Yield: 1.4 mg, 0.9 µmol, 6%.

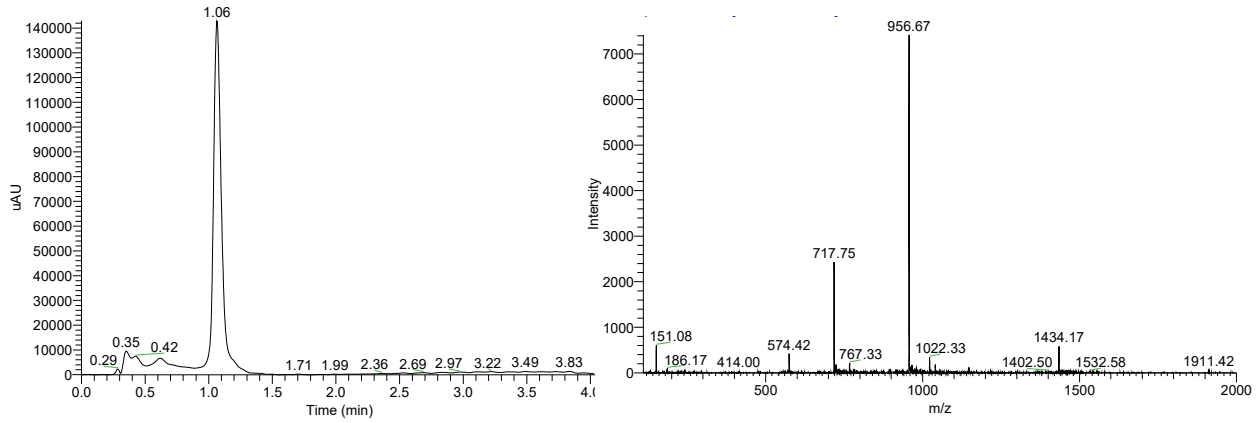


Figure S13. Analytical UPLC trace of HPLC purified **10**; R_t 1.06 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+2H]^{2+}$: 1434.44, $[M+3H]^{3+}$ 956.29; Mass Found (ESI+): $[M+2H]^{2+}$ 1434.17, $[M+3H]^{3+}$ 956.67.

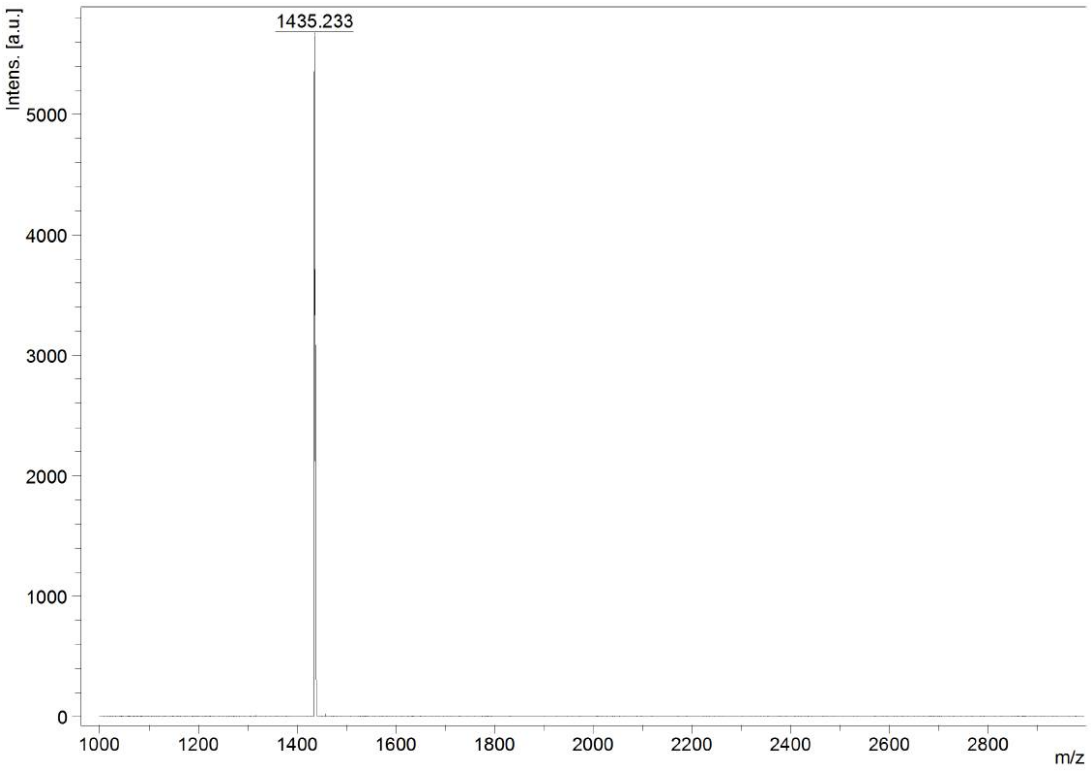
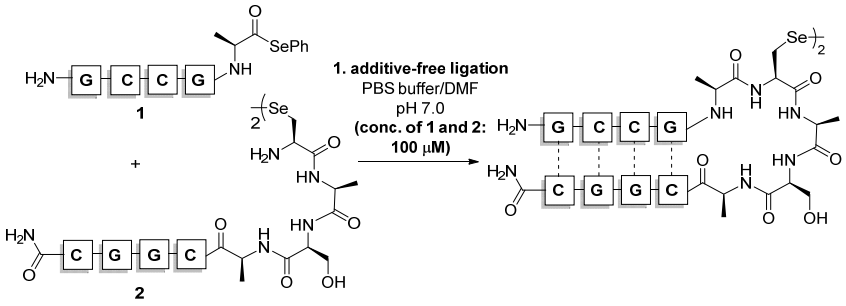


Figure S14. MALDI-TOF spectrum of HPLC purified **10**; Calculated *m/z* 1434.44 $[M+H]^+$, found 1435.233 $[M+H]^+$.

Model PNA-Templated Selenium-mediated Ligation Experiments

Ligation of PNA-peptide selenoester 1 and PNA-peptide diselenide dimer 2



The ligation of selenoester **1** (0.30 μmol) and diselenide dimer **2** (0.14 μmol) was performed as outlined in the general methods section at a concentration of 100 μM with respect to the diselenide dimer fragment (calculated based on the monomeric mass). After 1 minute, a 20 μL aliquot was removed for LCMS analysis, which confirmed conversion to the symmetrical diselenide ligation product.

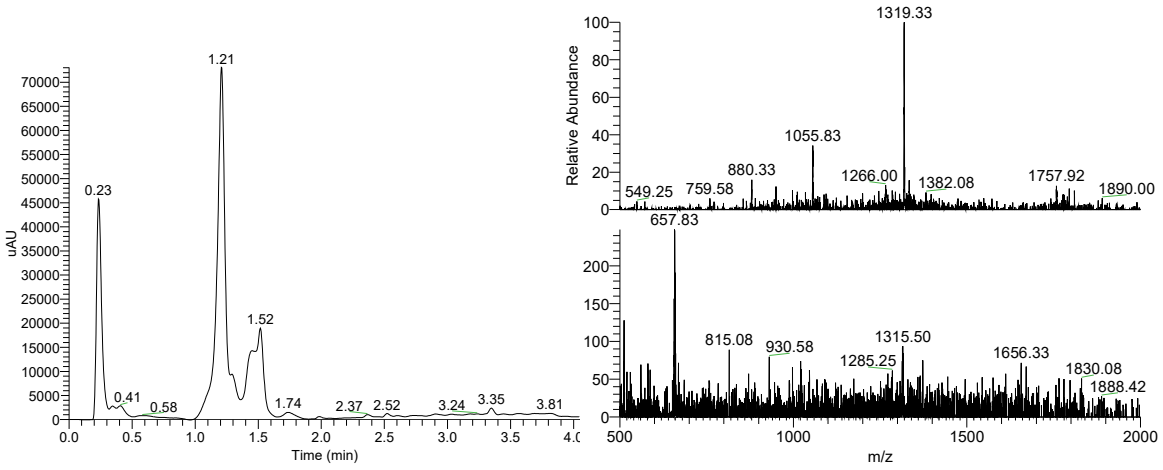
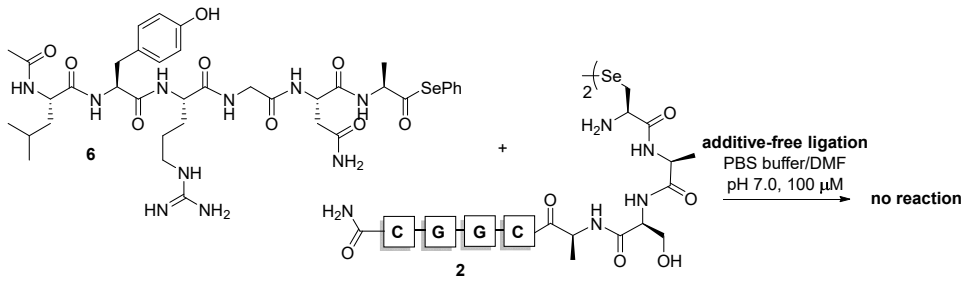
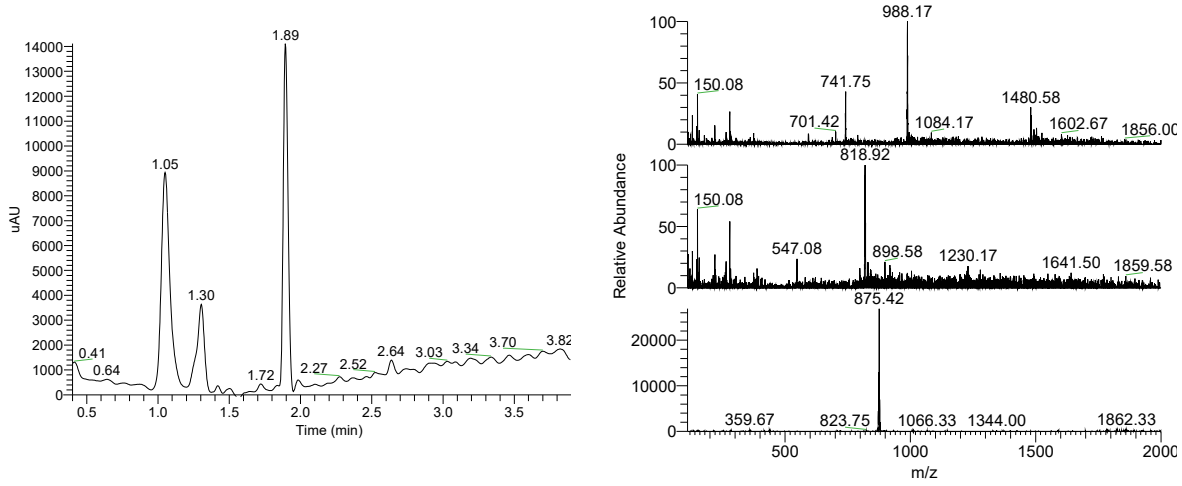


Figure S14. Analytical UPLC trace of crude reaction between **1** and **2** at 1 minute; Symmetrical Diselenide Ligation Product R_t 1.21 min (5-95% B over 4 min, λ = 280 nm) Calculated Mass $[M+3H]^{3+}$ 1759.02, $[M+4H]^{4+}$ 1319.52, $[M+5H]^{5+}$ 1055.81, $[M+6H]^{6+}$ 880.01; Mass Found (ESI+): $[M+3H]^{3+}$ 1757.92, $[M+4H]^{4+}$ 1319.33, $[M+5H]^{5+}$ 1055.83, $[M+6H]^{6+}$ 880.33; Selenoester **1** R_t 1.52 min (5-95% B over 4 min, λ = 280 nm) Calculated Mass $[M+H]^{+}$ 1314.19, $[M+2H]^{2+}$ 657.60; Mass Found (ESI+): $[M+H]^{+}$ 1315.50, $[M+2H]^{2+}$ 657.83.

Ligation of PNA-peptide Diselenide Dimer 2 and Peptide Selenoester 6



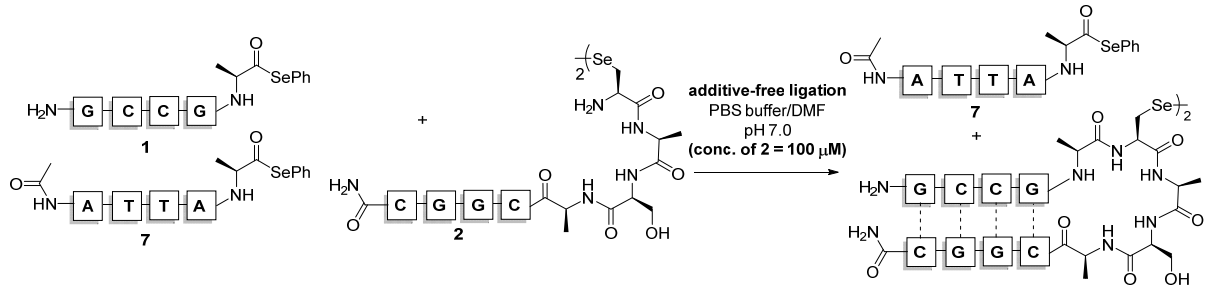
The ligation of peptide selenoester **6** (0.18 μmol) and diselenide dimer **2** (0.092 μmol) was performed as outlined in the general methods section at a concentration of 100 μM with respect to the diselenide dimer fragment (calculated based on the monomeric mass). After 1 h, a 20 μL aliquot was removed for LCMS analysis, which showed only starting materials remaining, together with some hydrolysis of



selenoester **6** (after 24 h).

Figure S15. Analytical UPLC trace of crude reaction between **2** and **6**; Diselenide **2** R_t 1.05 min (5-95% B over 4 min, λ = 280 nm) Calculated Mass $[M+2H]^{2+}$: 1481.84, $[M+3H]^{3+}$ 988.23; Mass Found (ESI+): $[M+2H]^{2+}$ 1480.58, $[M+3H]^{3+}$ 988.17, $[M+4H]^{4+}$ 741.75; Selenoester **6** R_t 1.89 min (5-95% B over 4 min, λ = 280 nm) Calculated Mass $[M+H]^{+}$: 874.87; Mass Found (ESI+): $[M+H]^{+}$ 875.42.

Ligation of PNA-peptide Diselenide Dimer 2 and PNA-peptide Selenoesters 1 and 7



The competitive ligation between dimer **2** (0.65 μmol) and selenoesters **1** and **7** (130 μmol, 10 eq.) was performed as outlined in the general methods section at a concentration of 100 μM with respect to the diselenide dimer fragment (calculated based on the monomeric mass). After 5 mins, a 20 μL aliquot was removed for LCMS analysis, which confirmed complete reaction of diselenide **2** with complimentary selenoester **1** to produce ligation product and unreacted non-complementary selenoester **7**.

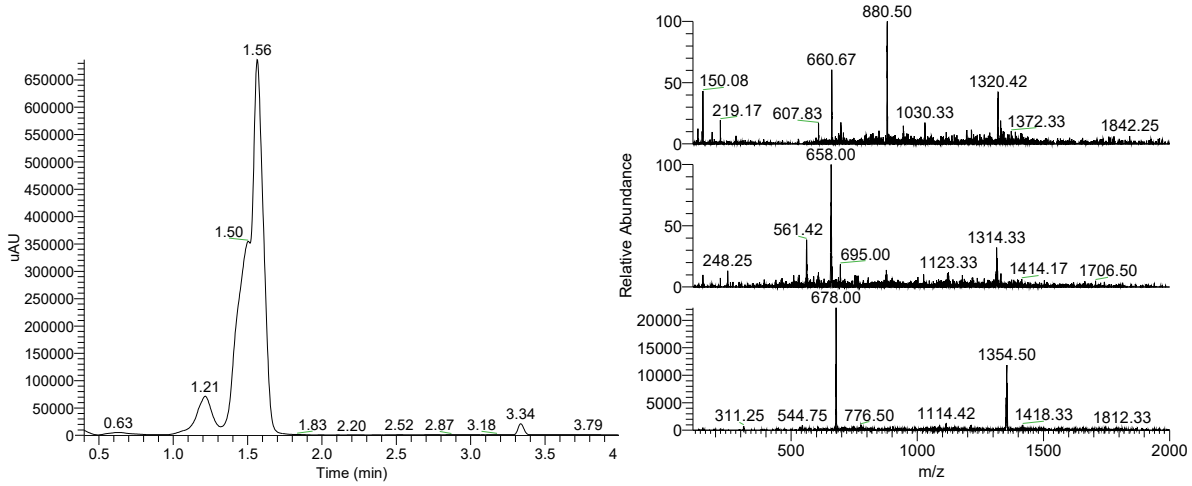
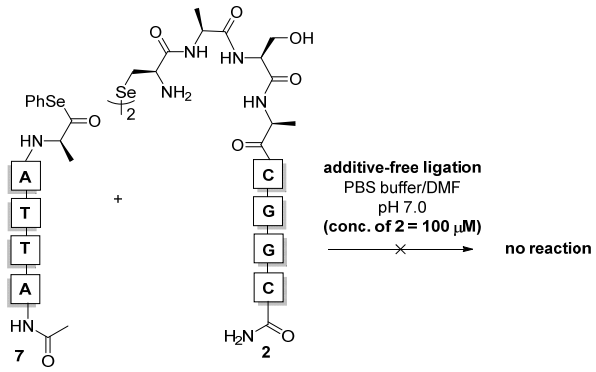


Figure S16. Analytical UPLC trace of crude reaction between diselenide dimer **2** and selenoesters **1** and **7**; Diselenide **2** and Selenoester **7** Ligation Product R_t 1.21 min (5-95% B over 4 min, λ = 280 nm) Calculated Mass $[M+2H]^{2+}$ 1319.52, $[M+3H]^{3+}$ 879.34, $[M+4H]^{4+}$ 659.76; Mass Found (ESI+): $[M+2H]^{2+}$ 1320.42, $[M+3H]^{3+}$ 880.50, $[M+4H]^{4+}$ 660.67; Excess selenoester **1** R_t 1.50 min (5-95% B over 4 min, λ = 280 nm) Calculated Mass $[M+H]^{+}$ 1314.19, $[M+2H]^{2+}$ 657.60; Mass Found (ESI+): $[M+H]^{+}$ 1314.33, $[M+2H]^{2+}$ 658.00; Unreacted selenoester **7** R_t 1.56 min (5-95% B over 4 min, λ = 280 nm) Calculated Mass $[M+H]^{+}$ 1354.44, $[M+2H]^{2+}$ 678.22; Mass Found (ESI+): $[M+H]^{+}$ 1354.50, $[M+2H]^{2+}$ 678.00.

Ligation of PNA-peptide Diselenide Dimer 2 and PNA-peptide Selenoester 7



The ligation of PNA selenoester **7** and diselenide dimer **2** was performed as outlined in the general methods section at a concentration of 100 μM with respect to the diselenide dimer fragment (calculated based on the monomeric mass). After 30 mins, a 20 μL aliquot was removed for LCMS analysis, which showed only starting materials remaining, together with some hydrolysis of selenoester **7**.

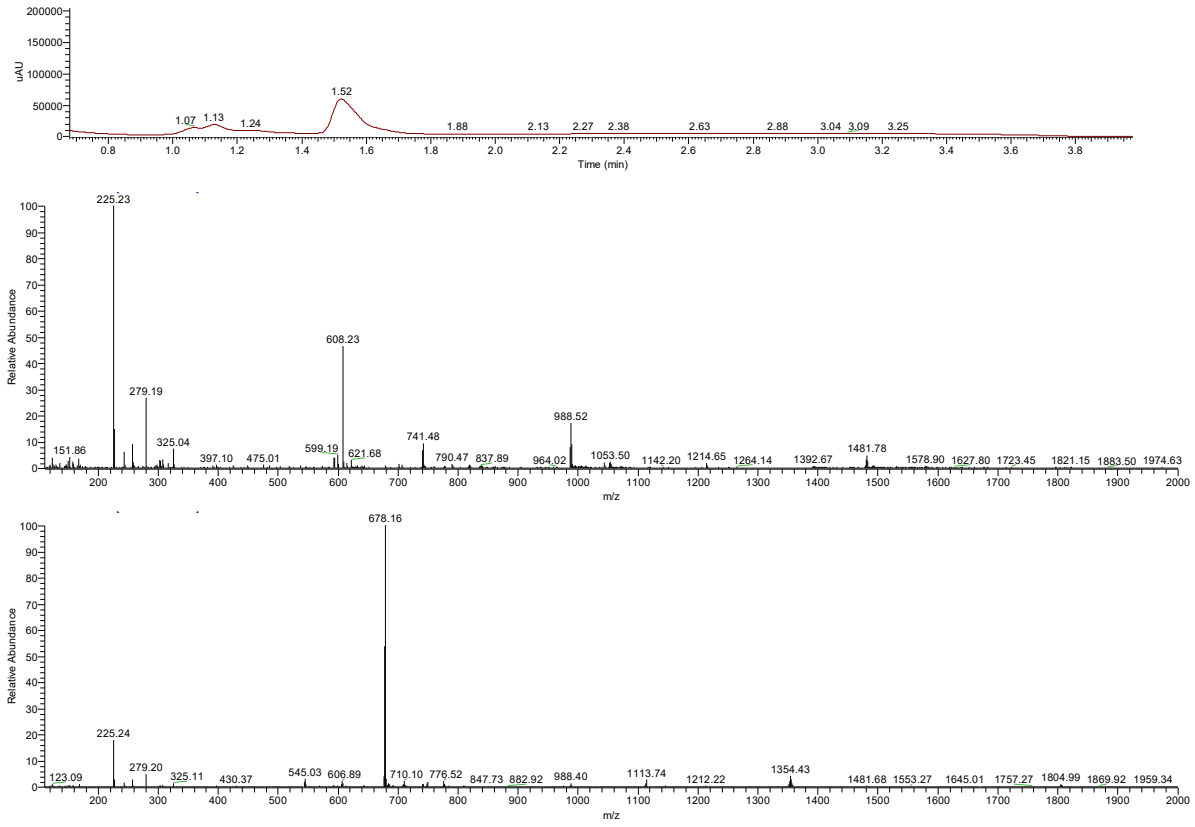


Figure S17. Analytical UPLC trace of crude reaction between diselenide dimer **2** and selenoester **7**; Diselenide **2** R_t 1.07 min (5-95% B over 4 min, λ = 280 nm) Calculated Mass $[M+2H]^{2+}$: 1481.84, $[M+3H]^{3+}$ 988.23; Mass Found (ESI+): $[M+2H]^{2+}$ 1481.78, $[M+3H]^{3+}$ 988.52, $[M+4H]^{4+}$ 741.48; Selenoester **7** R_t 1.52 min (5-95% B over 4 min, λ = 280 nm) Calculated Mass $[M+H]^{+}$ 1354.44, $[M+2H]^{2+}$ 678.22; Mass Found (ESI+): $[M+H]^{+}$ 1354.43, $[M+2H]^{2+}$ 678.16.

Kinetics

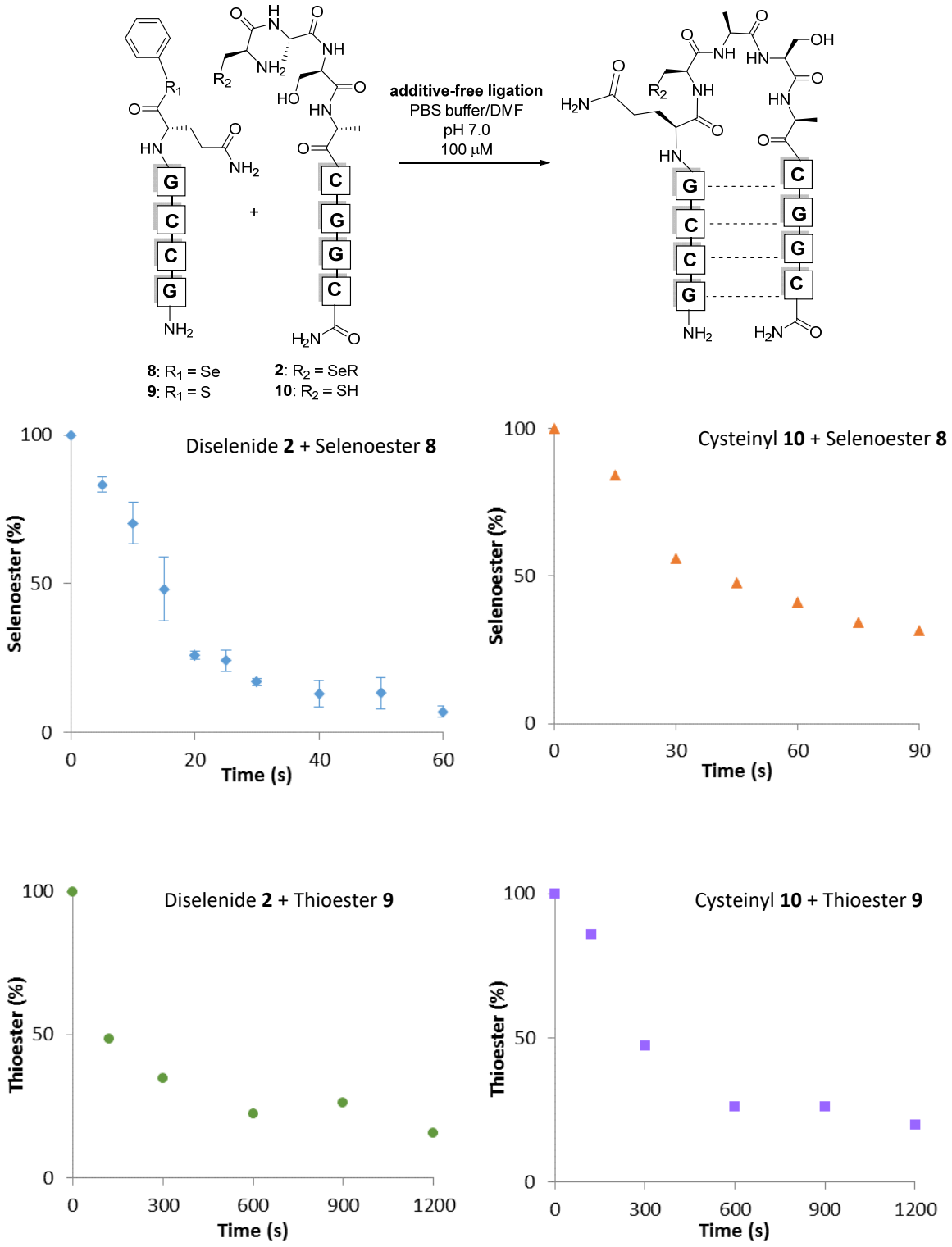


Figure S18. Integrated area of Selenoester **8** (Figure S18) or Thioester **9** (Figure S19) peak plotted against time over the course of the ligation reaction

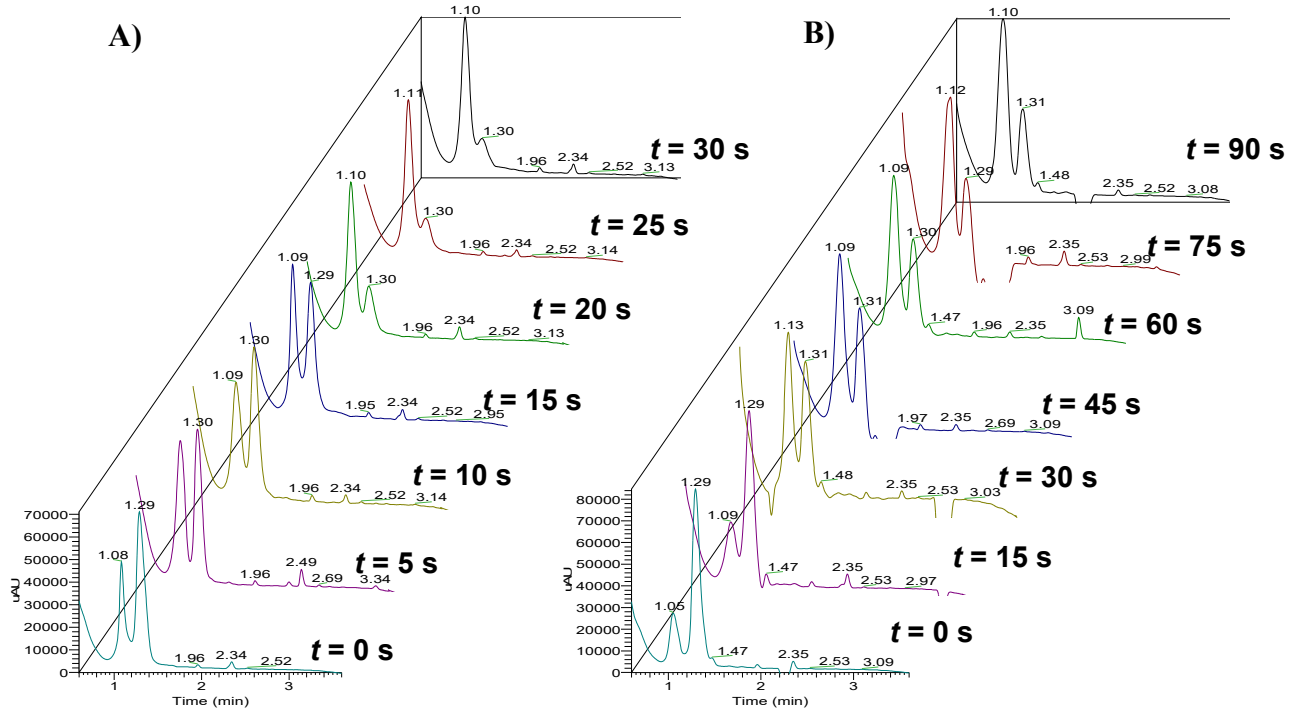


Figure S19. Analytical UPLC traces of crude reactions between **A)** Diselenide **2** and Selenoester **8** at timepoints 0-30 s and **B)** Cysteinyl peptide **10** and Selenoester **8** at timepoints 0-90 s; Selenoester **8** (R_t 1.30 min) is shown to be consumed over time as Ligation Product (R_t 1.10 min) is formed.

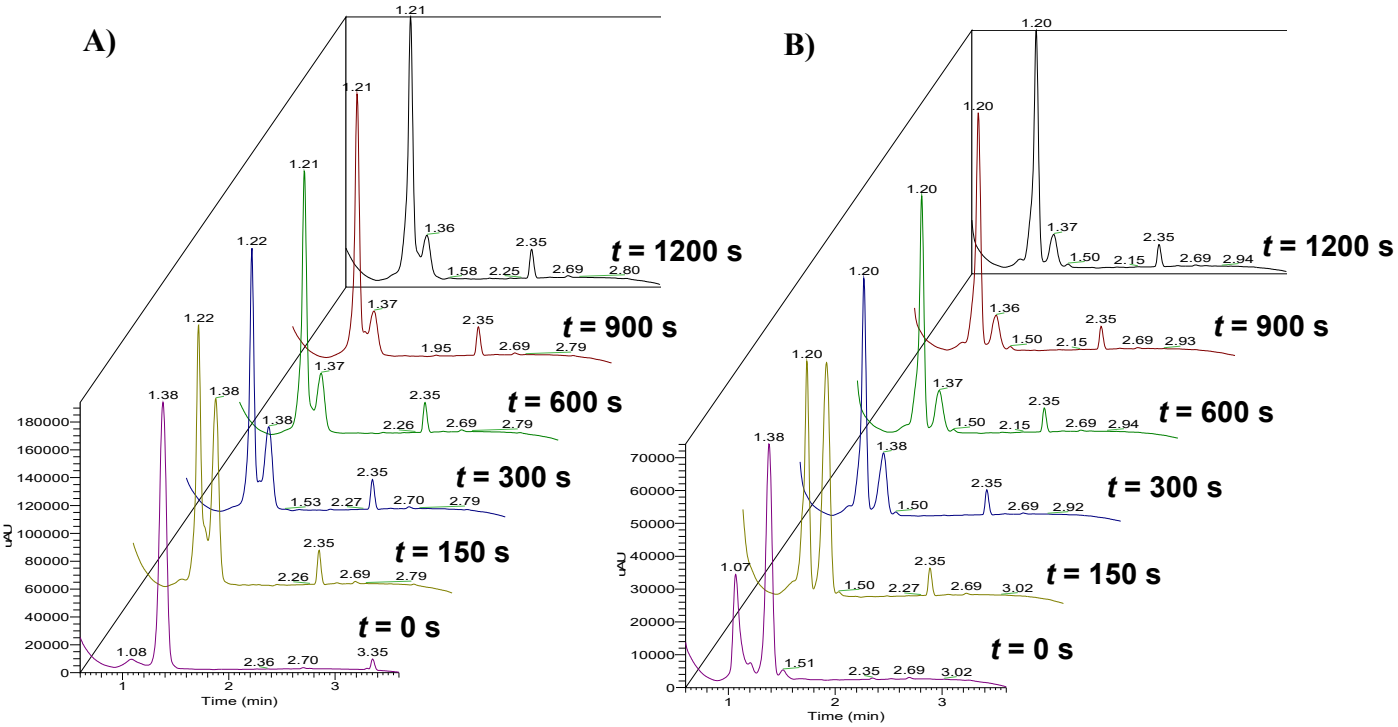
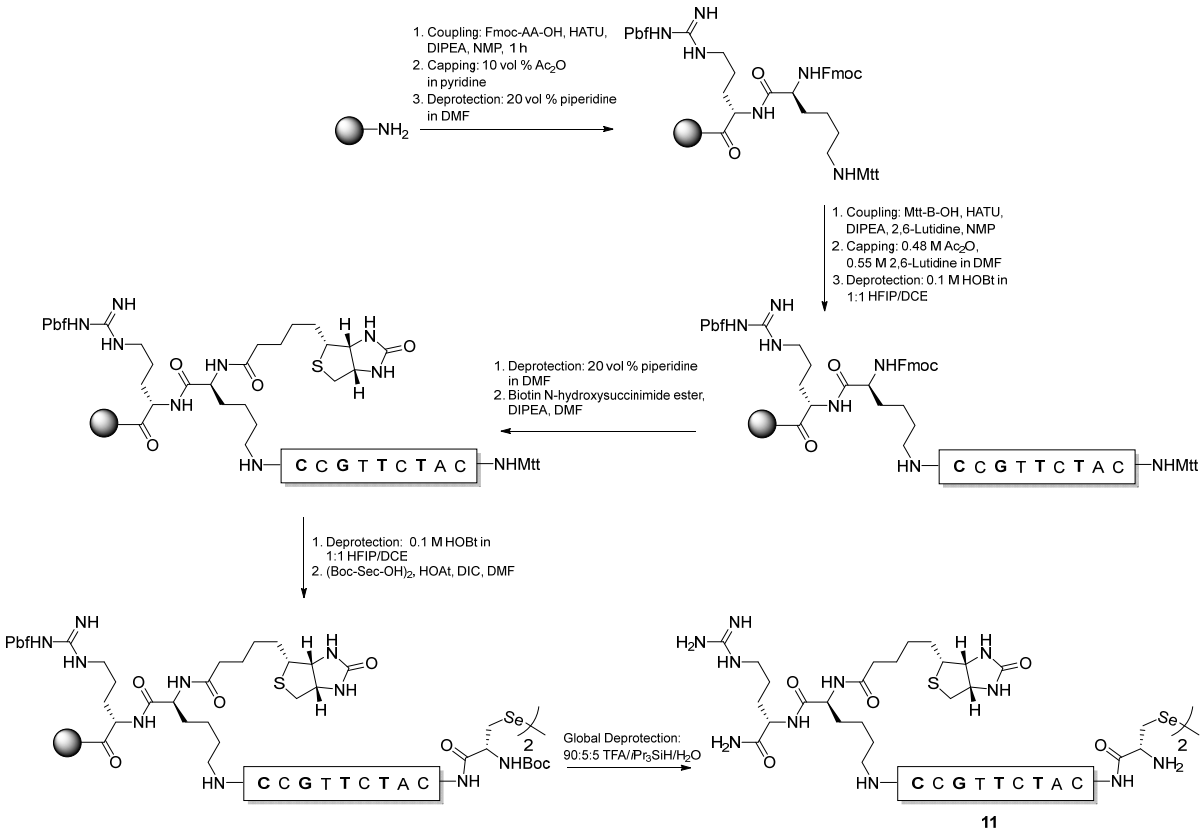


Figure S20. Analytical UPLC traces of crude reactions between **A)** Diselenide **2** and Thioester **9** and **B)** Cysteinyl peptide **10** and Thioester **9**, at timepoints 0-1200s; Thioester **9** (R_t 1.38 min) is shown to be consumed over time as Ligation Product (R_t 1.21 min) is formed.

Detection of miRNA

Synthesis of miR31 Detection Probes

Biotin labelled diselenide dimer miR-31 PNA probe (11)



To NovaPEG Rink amide resin (50 mg, 0.5 mmol/g, 25 μmol), Fmoc-Arg(Pbf)-OH (48.7 mg, 75 μmol, 3 eq.) was loaded as outlined in the general coupling procedure, followed by Fmoc-Lys(Mtt)-OH (46.9 mg, 75 μmol, 3 eq.). The Mtt side-chain protecting group of Lysine was then removed by treatment of the resin with 1:1 v/v HFIP/DCE containing 0.1 M HOBt. The resin was then split into two 12.5 μmol portions, one of which was elongated from the side-chain *via* automated Mtt-SPPS with PNA monomers (NB: each second PNA monomer was Serine-modified as shown by emboldened residues in synthetic scheme). With the N-terminal Mtt protecting group of the final PNA monomer intact, the Fmoc group of Lysine was deprotected and biotin N-hydroxysuccinimide ester (6.4 mg, 18.75 μmol, 1.5 eq.) was coupled as a solution in DMF with DIPEA (4.35 μL, 25.0 μmol, 2.0 eq.). The resin was then treated with 1:1 v/v HFIP/DCE containing 0.1 M HOBt to remove the Mtt group from the N-terminus of the PNA sequence. Coupling of $(Boc\text{-}Sec\text{-}OH)_2$ was then effected by treatment of the resin with a pre-activated solution of $(Boc\text{-}Sec\text{-}OH)_2$ (6.7 mg, 1.0 eq., 12.5 μmol), HOAt (42 μL, 2 eq., 25 μmol) and DIC (3.9 μL, 2 eq., 25 μmol) in NMP (final concentration 0.1 M). After 6 h of agitation at room temperature, the resin was washed with DMF (5 x 3 mL), CH_2Cl_2 (5 x

3mL) and DMF (5 x 3 mL). The fully extended PNA-peptide was cleaved from the solid support by treatment with an acidic cocktail of TFA/*i*Pr_3SiH/H_2O (95:2.5:2.5, v/v/v, 3 mL) for 1 h and then washed with TFA (2 x 2 mL). Crude **11** was precipitated from cold Et_2O and purified by reverse-phase semi-preparative HPLC (5-30% B over 12 min, 0.1 % TFA) to yield diselenide dimer **11** following lyophilisation. Calculated Diselenide M_W 6342.52; Yield: 1.6 mg, 0.5 µmol, 2%.

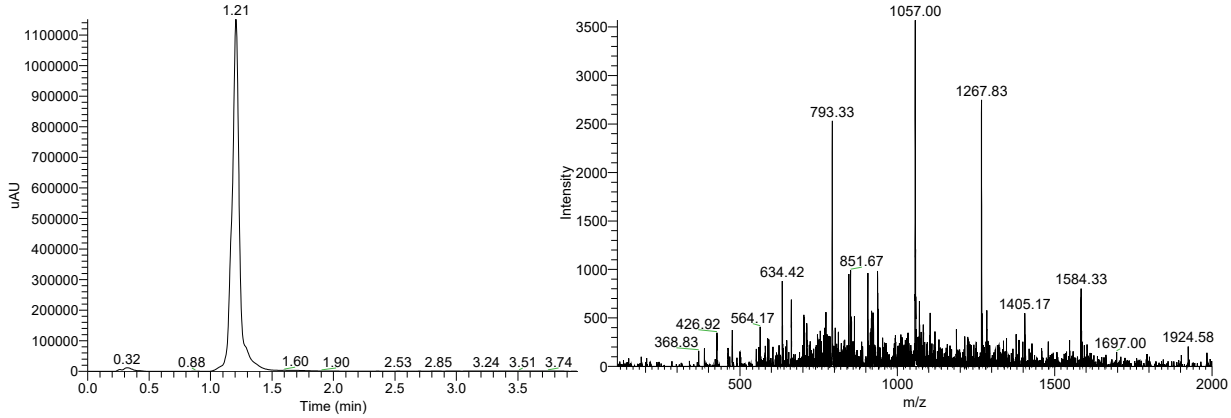


Figure S21. Analytical UPLC trace of HPLC purified **11**; R_t 1.21 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+4H]^{4+}$ 1586.63, $[M+5H]^{5+}$ 1269.50, $[M+6H]^{6+}$ 1058.10; Mass Found (ESI+): $[M+4H]^{4+}$ 1584.33, $[M+5H]^{5+}$ 1267.83, $[M+6H]^{6+}$ 1057.00.

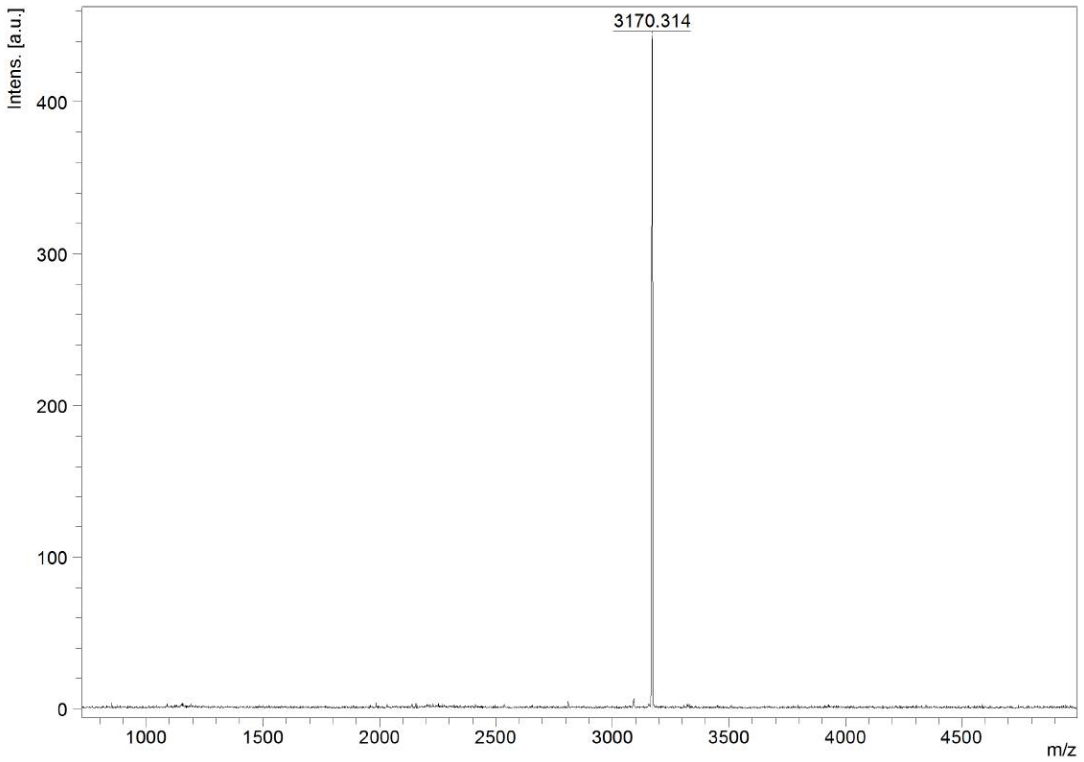
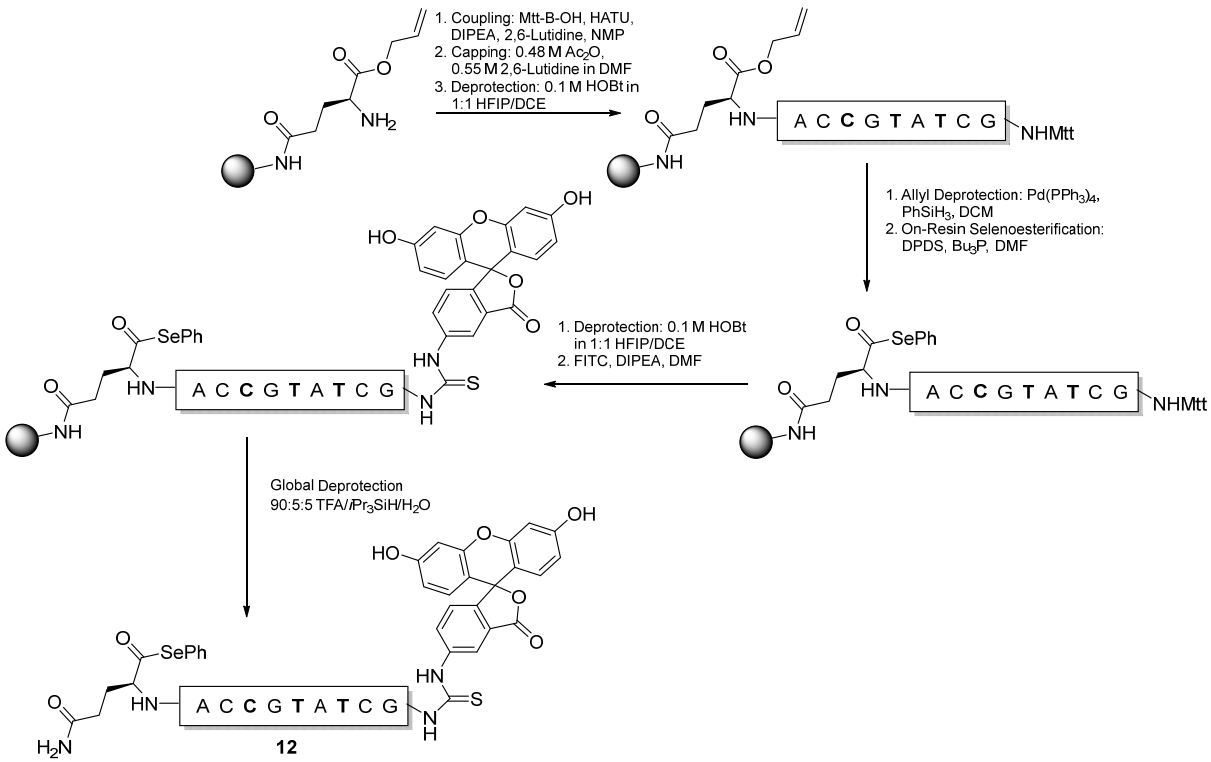


Figure S22. MALDI-TOF spectrum of HPLC purified **11**; Calculated Selenol *m/z* 3171.26 $[M+H]^+$, found 3170.314 $[M+H]^+$.

FITC-labelled selenoester miR-31 PNA probe (12)



NovaPEG Rink amide resin (25 mg, 0.5 mmol/g, 12.5 μmol) was loaded with Fmoc-Glu-OAll (5.1 mg, 1.0 eq., 12.5 μmol) as outlined in the general coupling procedure, and elongated with the PNA sequence *via* automated Mtt-SPPS (NB: bold residues indicate serine-modified PNA monomers). With the N-terminal PNA residue Mtt protected, the resin was then solvated in anhydrous CH_2Cl_2 for 5 minutes before $PhSiH_3$ (37 μL, 24 eq., 300 μmol) and $Pd(PPh_3)_4$ (3.6 mg, 0.25 eq., 3.13 μmol) were added in CH_2Cl_2, maintaining an inert atmosphere. The vessel was gently agitated at room temperature before washing with CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL). The resulting deprotected C-terminus was then selenoesterified on-resin as outlined in the general procedures. The N-terminal Mtt group was removed by treatment with 1:1 v/v HFIP/DCE containing 0.1 M HOBt. Fluoroscein isothiocyanate was dissolved in 200 μL NMP and DIPEA (4.4 μL, 25 μmol, 2.0 eq.) and transferred to the resin and gently agitated at room temperature for 30 mins. The resin was then washed with DMF (5 x 3 mL) and CH_2Cl_2 (5 x 3mL). Cleavage from the solid support was effected by treatment with an acidic cocktail (TFA/TIS/H_2O 95:2.5:2.5) for 30 mins and then concentrated under a stream of N_2. Following precipitation from Et_2O and purification by preparative HPLC, the desired selenoester **12** was obtained as a yellow solid after lyophilization. Calculated M_W 3187.03; Yield: 1.2 mg, 0.4 μmol, 3%.

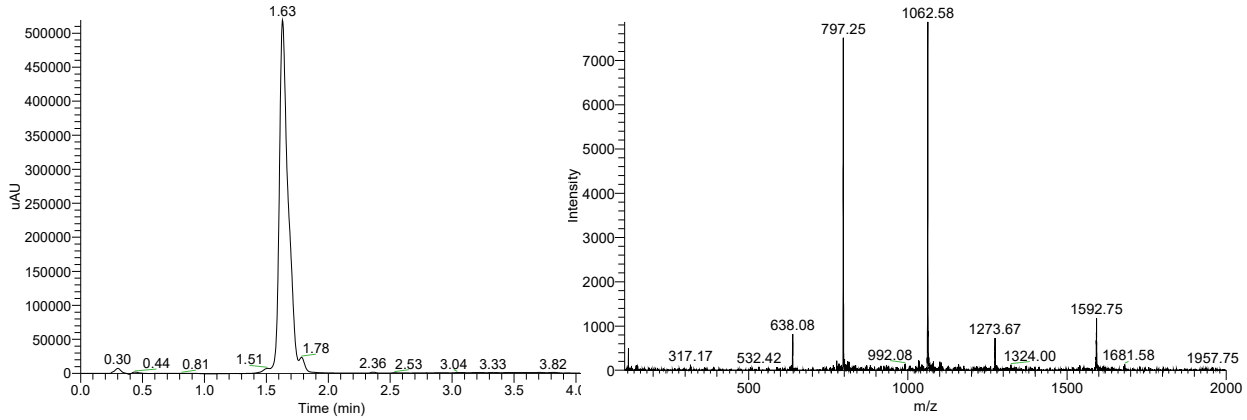


Figure S23. Analytical UPLC trace of HPLC purified **12**; R_t 1.63 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+2H]^{2+}$ 1593.03, $[M+3H]^{3+}$ 1062.35, $[M+4H]^{4+}$ 797.02; Mass Found (ESI+): $[M+2H]^{2+}$ 1592.75, $[M+3H]^{3+}$ 1062.58, $[M+4H]^{4+}$ 797.25.

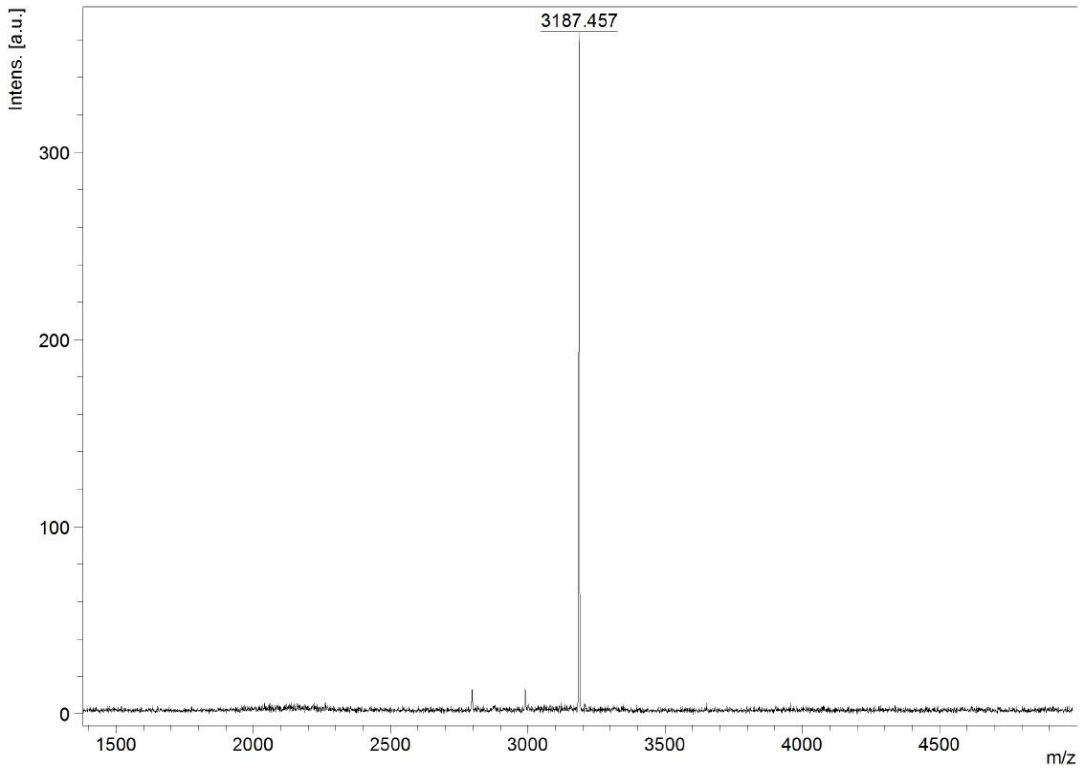


Figure S24. MALDI-TOF spectrum of HPLC purified **12**; Calculated *m/z* 3187.03 $[M+H]^+$, found 3187.457 $[M+H]^+$.

Ligation of Diselenide Dimer Probe 11 and Selenoester Probe 12

The ligation of diselenide dimer probe **11** and selenoester probe **12** was performed as outlined in the general methods section at a concentration of 100 μM with respect to the diselenide dimer fragment (calculated based on monomeric mass), in the presence of 10 μM purified miR-31 DNA template. A 20 μL aliquot was taken for LCMS analysis, which confirmed conversion to the ligation product as well as both starting materials remaining (due to excess of both probes present).

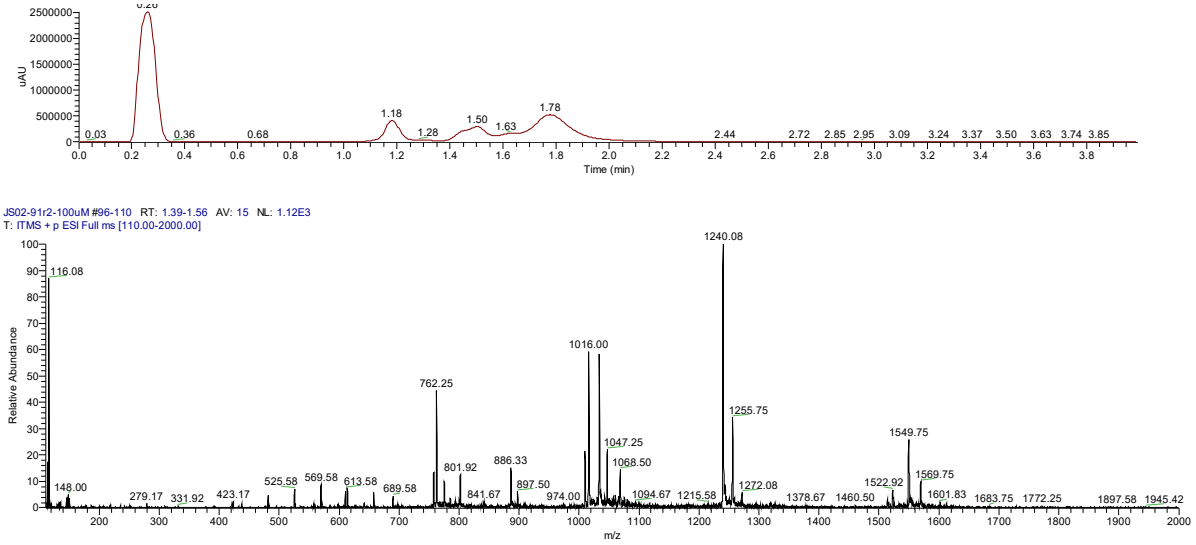
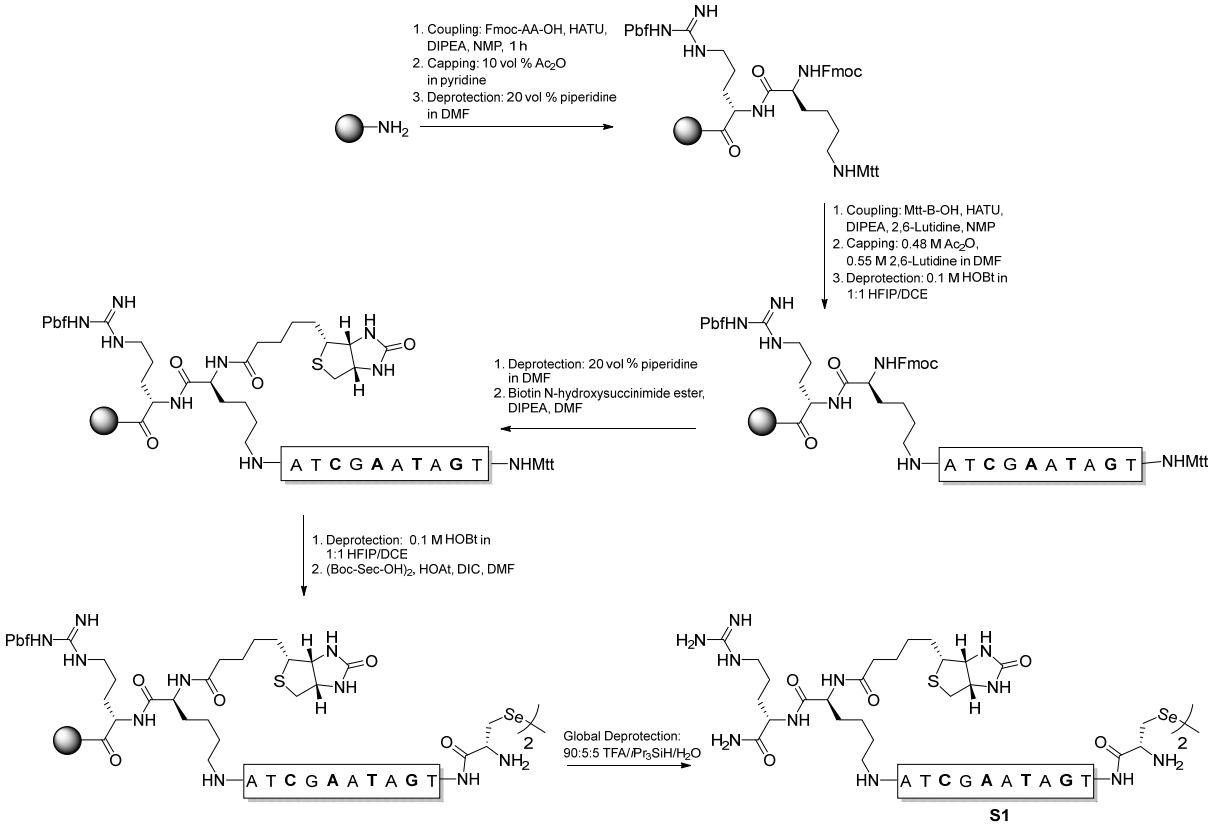


Figure S25. Analytical UPLC trace of crude reaction between diselenide dimer **11** and selenoester **12**; Ligation Product R_t 1.50 min (5-95% B over 4 min, λ = 280 nm) Calculated Mass $[M+4H]^{4+}$ 1549.59, $[M+5H]^{5+}$ 1239.67, $[M+6H]^{6+}$ 1033.06, $[M+7H]^{7+}$ 885.48; Mass Found (ESI+): $[M+4H]^{4+}$ 1549.75, $[M+5H]^{5+}$ 1240.08, $[M+6H]^{6+}$, $[M+7H]^{7+}$ 886.33; Excess diselenide **11** R_t 1.18 min (5-95% B over 4 min, λ = 280 nm); Excess selenoester **12** R_t 1.78 min (5-95% B over 4 min, λ = 280 nm).

Synthesis of miR21 Detection Probes

Biotin-labelled diselenide dimer miR-21 PNA probe (S1)



To NovaPEG Rink amide resin (50 mg, 0.5 mmol/g, 12.5 μmol), Fmoc-Arg(Pbf)-OH (24.4 mg, 37.5 μmol, 3 eq.) was loaded as outlined in the general coupling procedure, followed by Fmoc-Lys(Mtt)-OH (23.5 mg, 37.5 μmol, 3 eq.). The Mtt side-chain protecting group of Lysine was then removed by treatment of the resin with 1:1 HFIP/DCE containing 0.1 M HOBt. The resin was then elongated from the side-chain *via* automated Mtt-SPPS with PNA monomers (NB: each second PNA monomer was Serine-modified as shown by emboldened residues in synthetic scheme). With the N-terminal Mtt protecting group of the final PNA monomer intact, the Fmoc group of Lysine was deprotected and biotin N-hydroxysuccinimide ester (6.4 mg, 18.75 μmol, 1.5 eq.) was coupled as a solution in DMF with DIPEA (4.35 μL, 25.0 μmol, 2.0 eq.). The resin was then treated with 1:1 v/v HFIP/DCE containing 0.1 M HOBt to remove the Mtt group from the N-terminus of the PNA sequence. Coupling of $(Boc\text{-}Sec\text{-}OH)_2$ was then effected by treatment of the resin with a pre-activated solution of $(Boc\text{-}Sec\text{-}OH)_2$ (6.7 mg, 1.0 eq., 12.5 μmol), HOAt (42 μL, 2 eq., 25 μmol) and DIC (3.9 μL, 2 eq., 25 μmol) in NMP (final concentration 0.1 M). After 6 h of agitation at room temperature, the resin was washed with DMF (5 x 3 mL), CH_2Cl_2 (5 x 3mL) and DMF (5 x 3 mL). The fully extended PNA-peptide was cleaved from the solid support by treatment with an acidic cocktail of TFA/iPr$_3$SiH/H_2O (95:2.5:2.5, v/v/v, 3 mL) for 1 h and then washed with TFA (2 x 2 mL). Crude **S1** was precipitated

from cold Et_2O and purified by reverse-phase semi-preparative HPLC (5-30% B over 12 min, 0.1 % TFA) to yield diselenide dimer **S1** following lyophilisation. Calculated M_W 3537.37; Yield: 0.9 mg, 0.3 μmol, 2%.

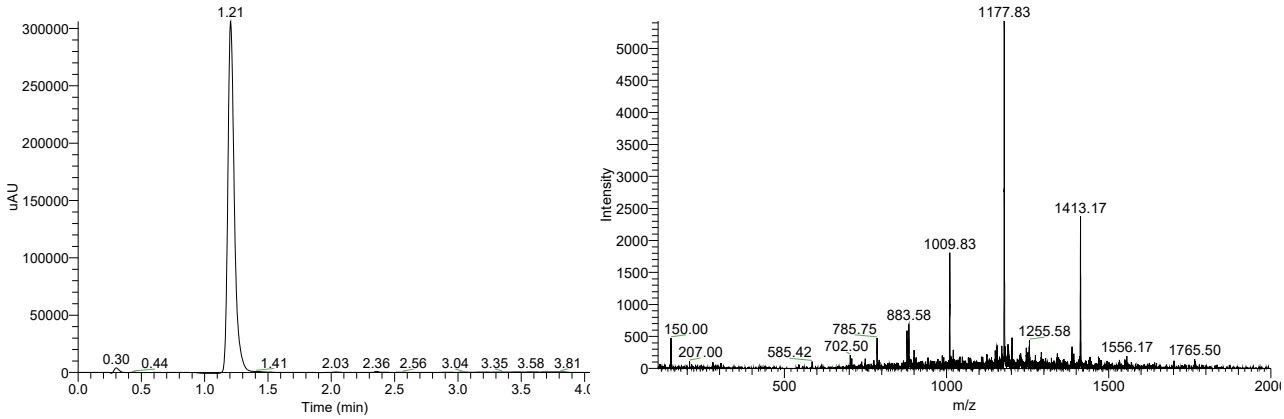


Figure S26. Analytical UPLC trace of HPLC purified **S1**; R_t 1.21 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+5H]^{5+}$ 1415.95, $[M+6H]^{6+}$ 1180.12, $[M+7H]^{7+}$ 1011.68; Mass Found (ESI+): $[M+5H]^{5+}$ 1413.17, $[M+6H]^{6+}$ 1177.83, $[M+7H]^{7+}$ 1009.83.

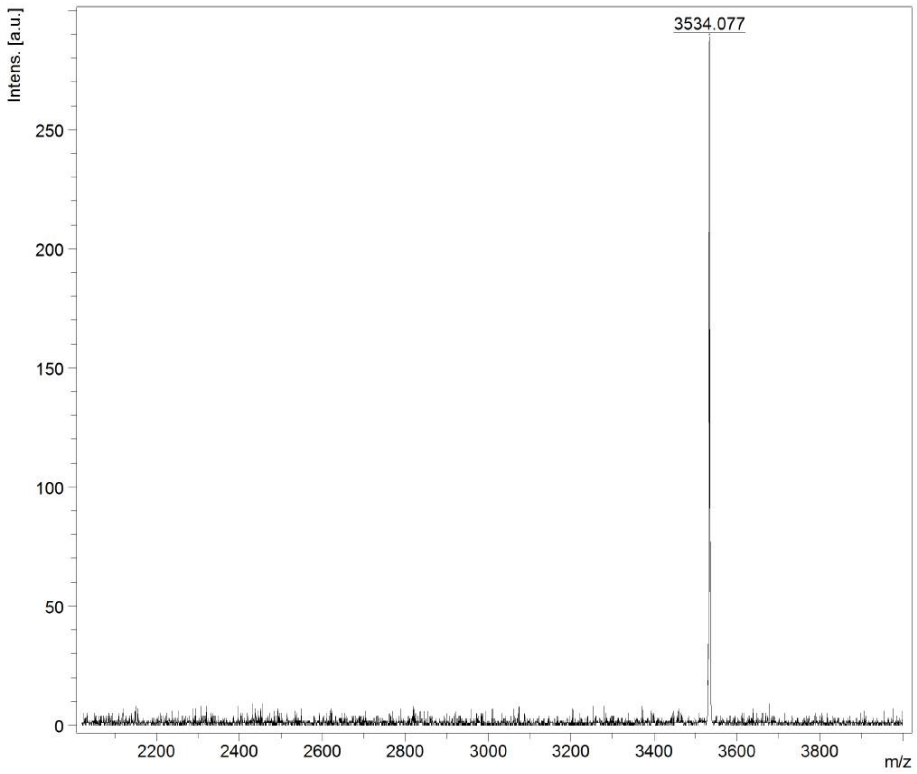
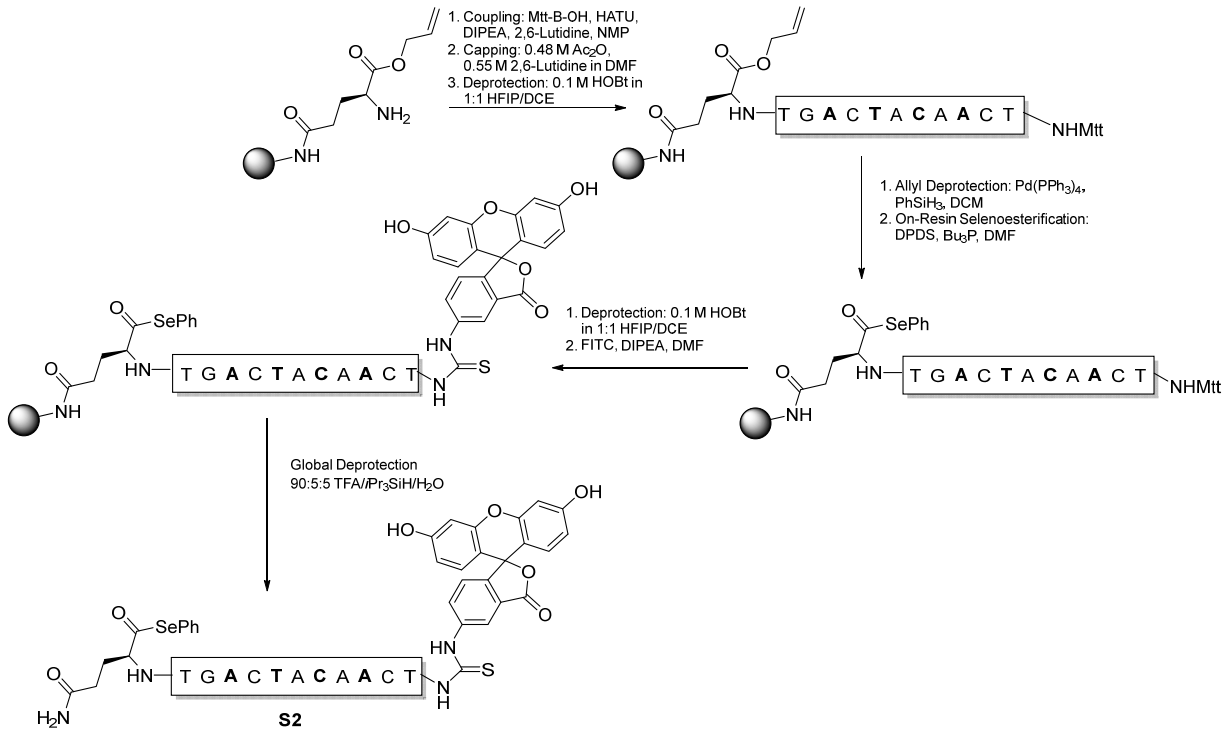


Figure S27. MALDI-TOF spectrum of HPLC purified **S1**; Calculated *m/z* 3536.60 $[M+H]^+$, found 3534.077 $[M+H]^+$.

FITC-labelled selenoester miR-21 PNA probe (S2)



NovaPEG Rink amide resin (25 mg, 0.5 mmol/g, 12.5 μmol) was loaded with Fmoc-Glu-OAll (5.1 mg, 1.0 eq., 12.5 μmol) as outlined in the general coupling procedure, and elongated with the PNA sequence *via* automated Mtt-SPPS (NB: bold residues indicate serine-modified PNA monomers). With the N-terminal PNA residue Mtt protected, the resin was then solvated in anhydrous CH_2Cl_2 for 5 minutes before $PhSiH_3$ (37 μL, 24 eq., 300 μmol) and $Pd(PPh_3)_4$ (3.6 mg, 0.25 eq., 3.13 μmol) were added in CH_2Cl_2, maintaining an inert atmosphere. The vessel was gently agitated at room temperature before washing with CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL). The resulting deprotected C-terminus was then selenoesterified on-resin as outlined in the general procedures. The N-terminal Mtt group was removed by treatment with 1:1 v/v HFIP/DCE containing 0.1 M HOBt. Fluoroscein isothiocyanate was dissolved in 200 μL NMP and DIPEA (4.4 μL, 25 μmol, 2.0 eq.) and transferred to the resin and gently agitated at room temperature for 30 mins. The resin was then washed with DMF (5 x 3 mL) and CH_2Cl_2 (5 x 3mL). Cleavage from the solid support was effected by treatment with an acidic cocktail (TFA/TIS/H_2O 95:2.5:2.5) for 30 mins and then concentrated under a stream of N_2. Following precipitation from Et_2O and purification by preparative HPLC, the desired selenoester **S2** was obtained as a yellow solid after lyophilisation. Calculated M_W 3741.29; Yield: 1.5 mg, 0.4 μmol, 3%.

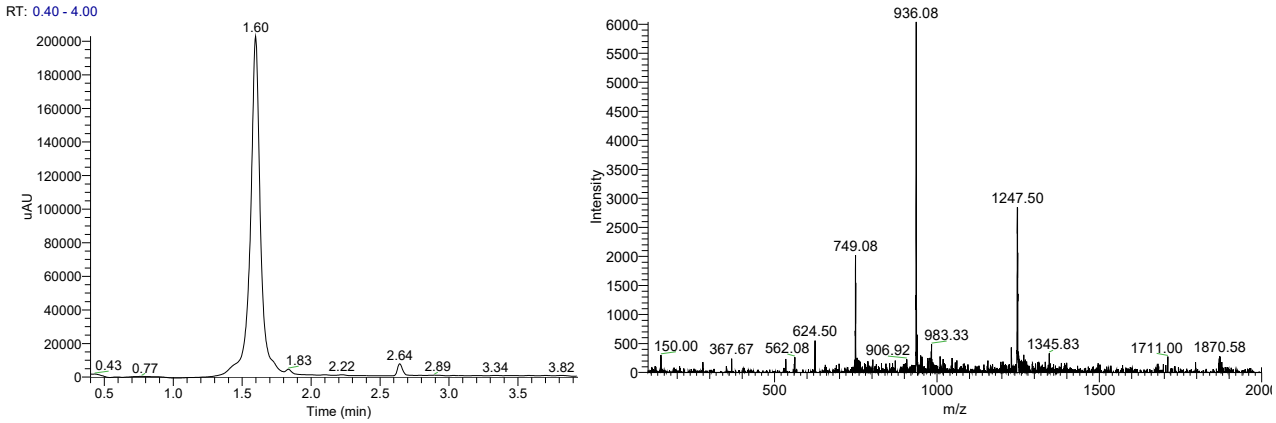


Figure S28. Analytical UPLC trace of HPLC purified **S2**; R_t 1.60 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+2H]^{2+}$ 1871.65, $[M+3H]^{3+}$ 1248.10, $[M+4H]^{4+}$ 936.32; Mass Found (ESI+): $[M+2H]^{2+}$ 1870.58, $[M+3H]^{3+}$ 1247.50, $[M+4H]^{4+}$ 938.08.

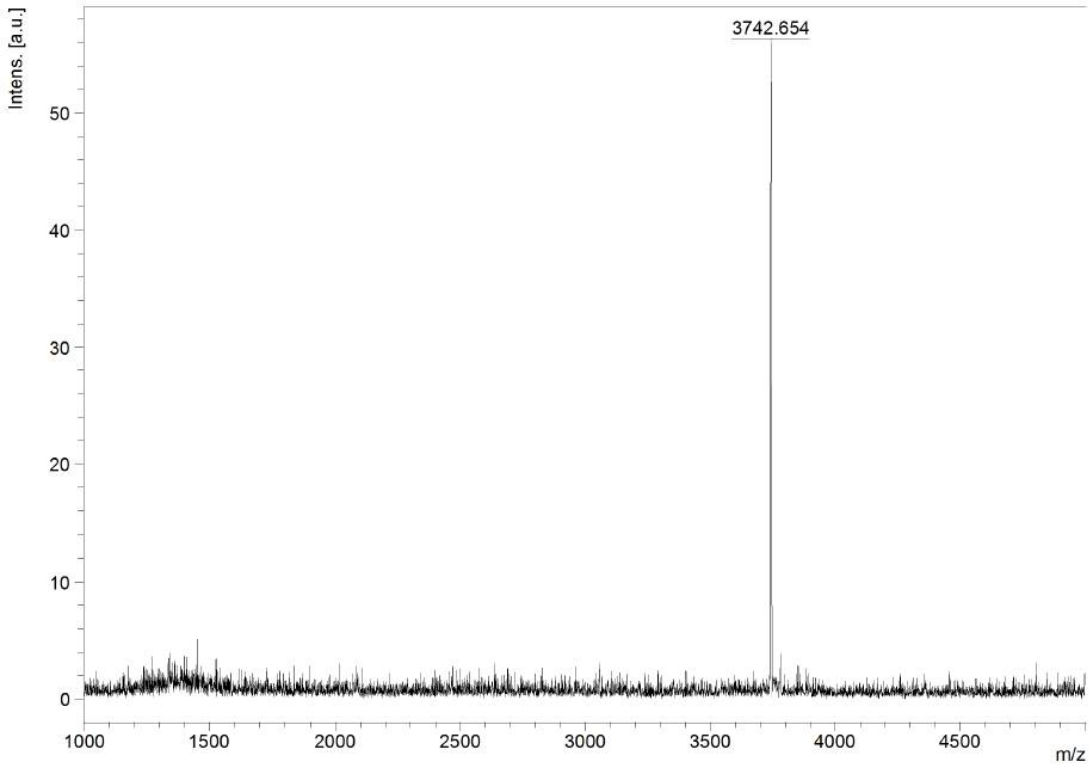


Figure S29. MALDI-TOF spectrum of HPLC purified **S2**; Calculated *m/z* 3742.29 $[M+H]^+$, found 3742.654 $[M+H]^+$.

Lateral Flow microRNA Detection Assay

DNA templates were purchased from Eurogentec as desalted 100 μM solutions in deionised water. Stock DNA solutions at 1 μM and 100 nM were prepared in PBS buffer pH 7.4 and stored at -20 °C. Stock solutions of PNA probes at 10 μM and 1 μM were prepared in anhydrous DMF and stored at -80 °C.

miR-31 DNA Template Sequences:

Template A (Match): **AGG CAA GAT GCT GGC ATA GCT**

Template B (Partial Mismatch): **AGG AAT GAT GCT GGC ATA GCT**

Template C (Mismatch): **TAG CTT ATC AGA CTG ATG TTG A**

Assay Procedure

A stock solution of PBS buffer containing 5μM TCEP was prepared at pH 7.0. Stock solutions of PNA probes **11** and **12** (10 μM or 1 μM) were removed from storage at -80 °C and thawed immediately prior to use. Aqueous buffer was aliquoted into an eppendorf tube and heated to the desired temperature for 5 minutes. The DNA analyte/template was added from a stock aqueous solution (1 μM and 100 nM) to the desired final concentration, followed by the diselenide probe then the selenoester probe in a 1:1 stoichiometric ratio. After brief agitation, the assay was allowed to proceed for precisely 2 minutes at the desired temperature. A Milenia® HybriDetect 2T test strip was inserted. The solution was allowed to migrate beyond the control zone and the strip was then removed and allowed to dry at room temperature. The results were evaluated visually and imaged for processing and quantification using ImageJ – an open source Java image processing program.

Negative Control Assays

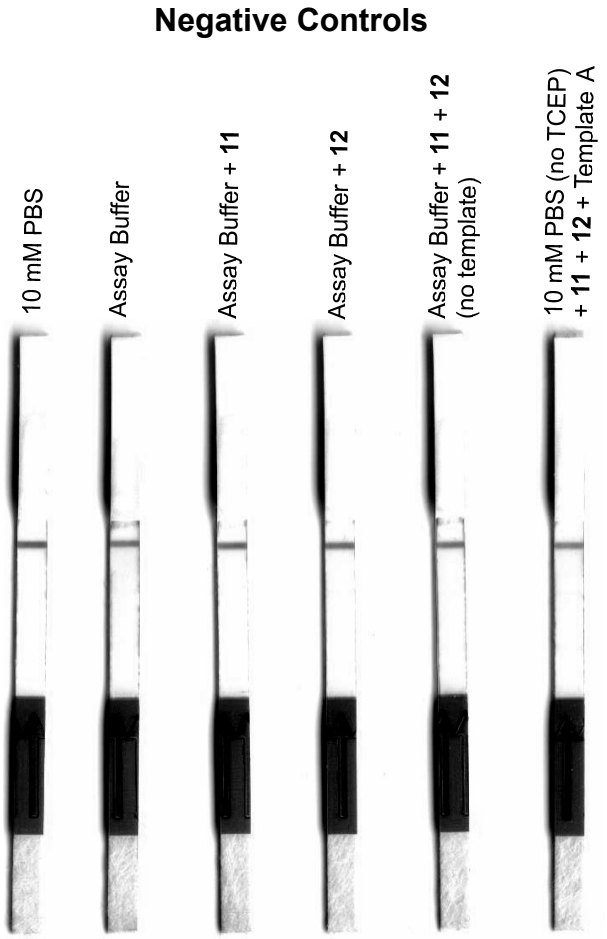


Figure S30. Test strip results of negative control assays. Assay buffer is 10 mM PBS containing 5 μM TCEP. Probes **11** and **12** were added as stock solutions in DMF to a final concentration of 10 nM. Template A was added to a final concentration of 1 nM.

Detection of miR-31 in the absence of Ligation

To confirm that the templated selenocystine-selenoester ligation is necessary for miRNA detection, we carried out the assay using diselenide dimer probe **11** and a variant of selenoester probe **12** hydrolysed to a carboxylic acid and therefore unable to participate in the ligation reaction. First, selenoester **12** was allowed to hydrolyse for 2 h as a 50 μM aqueous solution.

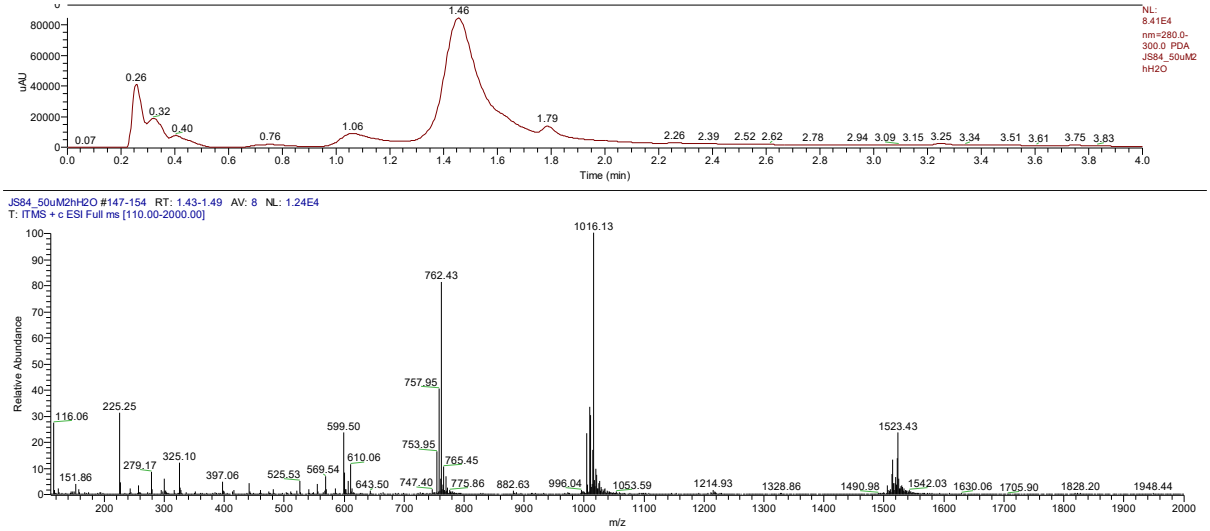


Figure S31. Analytical UPLC trace of crude hydrolysed-**12**; R_t 1.46 min (5-95% B over 4 min, λ = 280 nm); Calculated Mass $[M+2H]^{2+}$ 1523.05, $[M+3H]^{3+}$ 1015.7, $[M+4H]^{4+}$ 762.03; Mass Found (ESI+): $[M+2H]^{2+}$ 1523.43, $[M+3H]^{3+}$ 1016.13, $[M+4H]^{4+}$ 762.43.

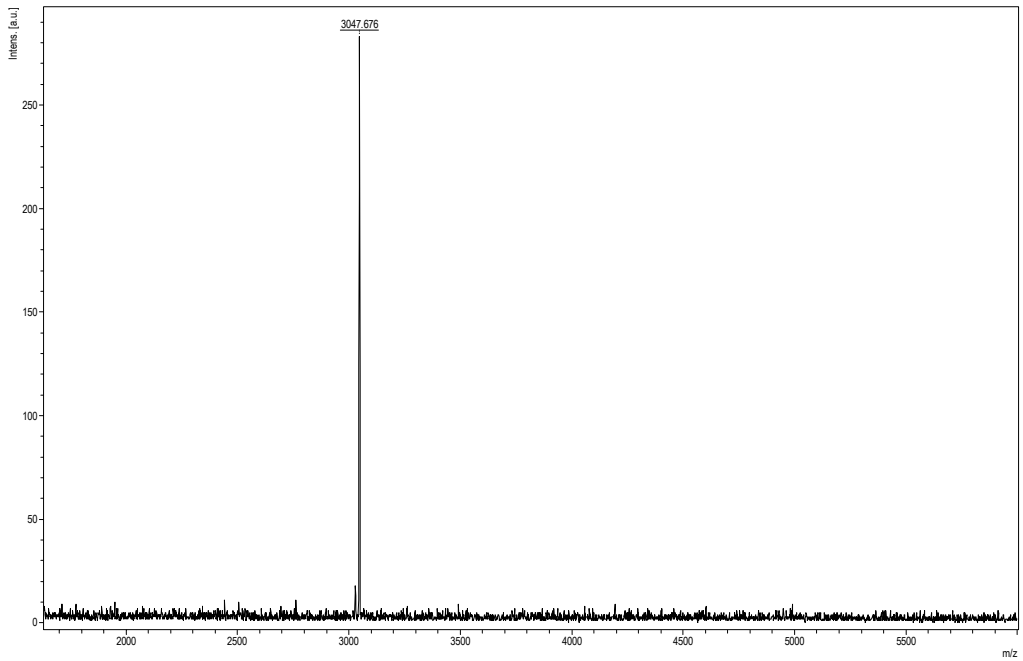
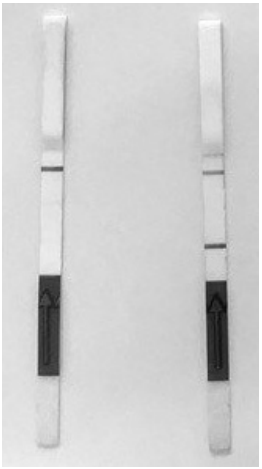


Figure S32. MALDI-TOF spectrum of hydrolysed-**12**; Calculated *m/z* 3047.1 $[M+H]^+$, found 3047.7 $[M+H]^+$.

The hydrolysed-**12** material was then used in the miR-31 detection assay with diselenide dimer probe **11** as outlined in the general assay procedure. The final concentration of both probes was 10 nM and the purified miR-31 DNA template was also present at 10 nM concentration. The test strip result was negative, as shown below alongside a positive control (probes **11** + **12**):

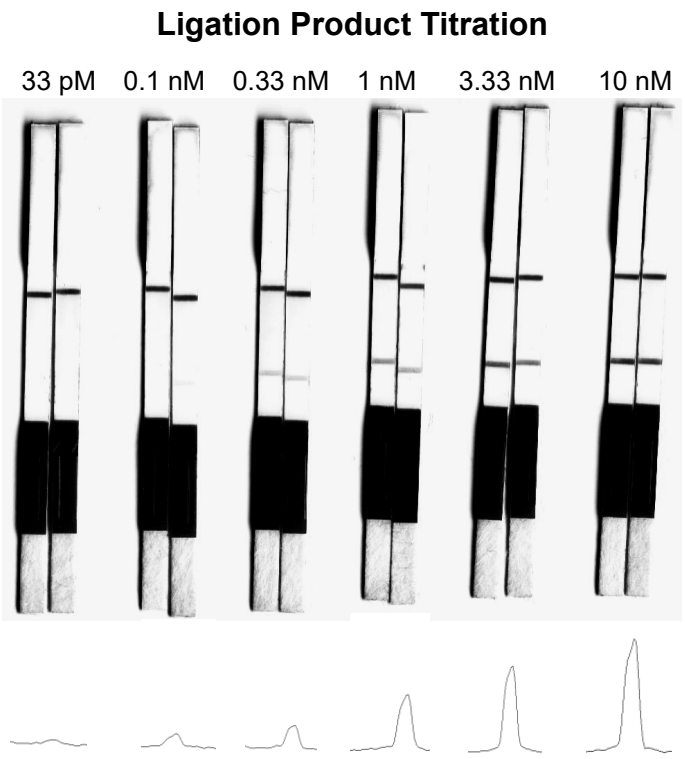
Probe **11** + Hydrolysed-**12**



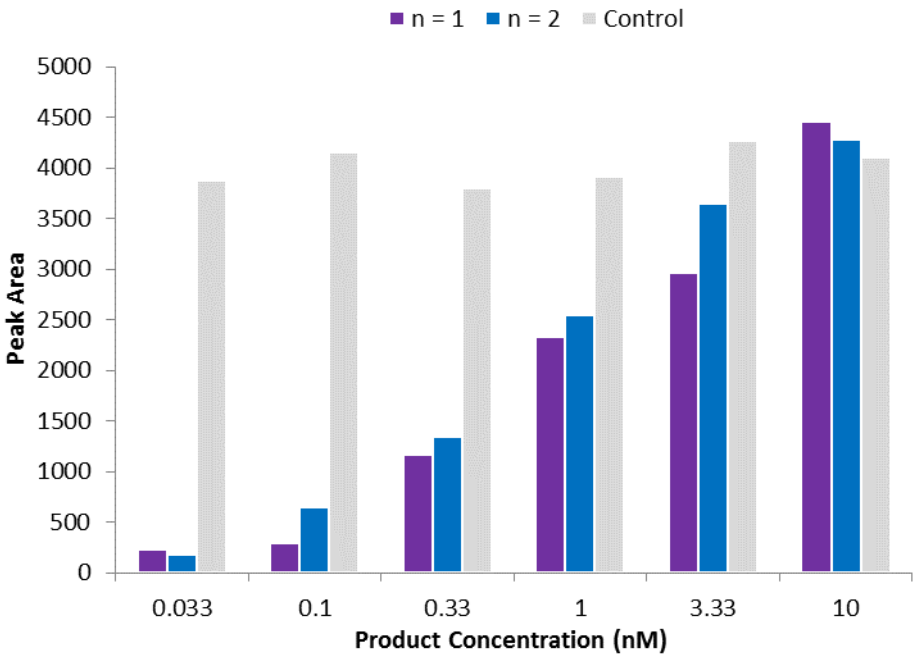
Probe **11** + Selenoester **12**

Detection of miR-31 in Model Systems

A



B



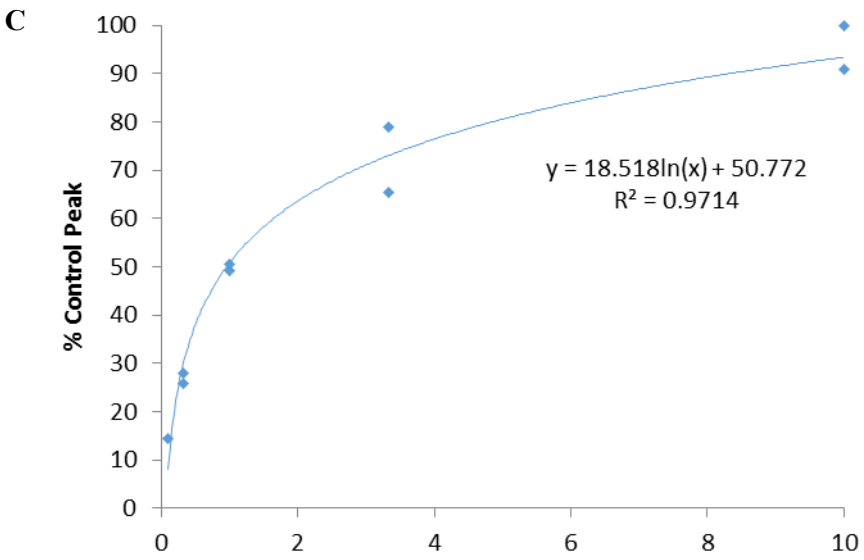
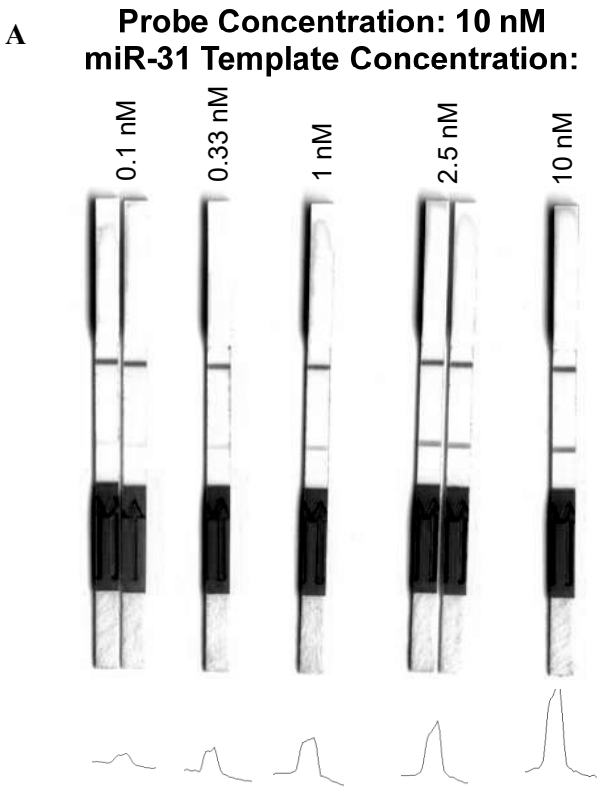
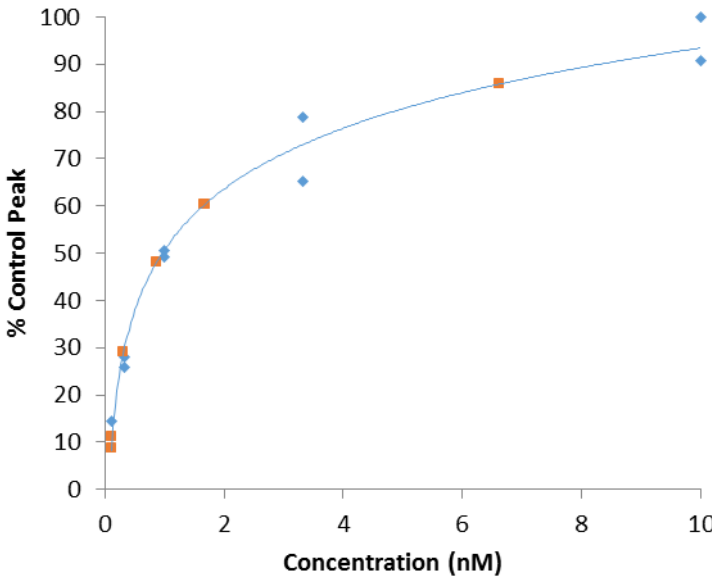


Figure S33. A. Test strip results of product titration, **B.** Integrated peak areas (ImageJ) of product titration test strips plotted against concentration and showing 10 nM saturation point and 0.1 nM lower limit of detection, **C**. Test strip signal intensities of ligation product titration plotted against concentration.

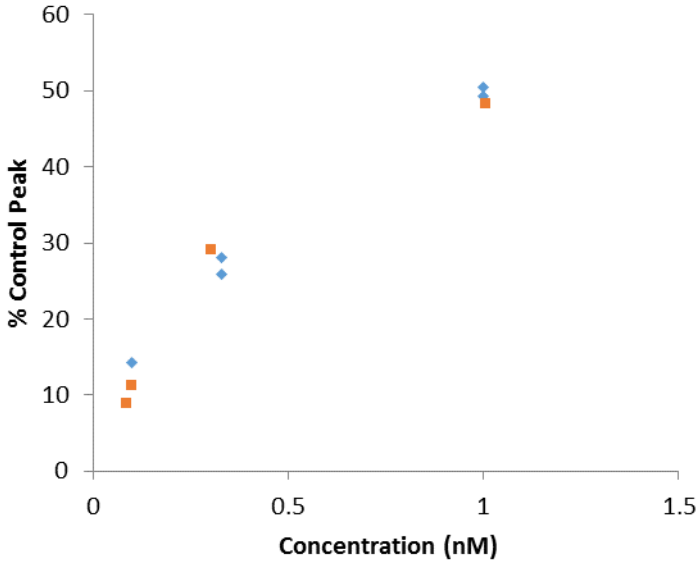
Quantification



B



C



D

Probe Concentration (nM)	miRNA Concentration (nM)	Calculated Product Concentration (nM)	Estimated Ligation Yield (%)
10	10	6.63	66
10	2.5	1.69	67
10	2.5	1.67	67
10	1	0.87	87
10	0.33	0.31	94
10	0.1	0.12	*Quant.*
10	0.1	0.10	*Quant.*

Figure S34. A. Test strip results of assay carried out at varied miR-31 template concentrations (10, 2.5, 1, 0.33 and 0.1 nM) and constant probe concentration (10 nM); **B.** Integrated peak areas (ImageJ) of test strips at known miR-31 concentration plotted on the standard product titration curve; **C.** Expanded region of plot **B** to show close correlation of measured signal intensities compared to the standard titration curve between 0.1 nM and 1 nM template concentration; **D.** Table of predicted values obtained from plot **B**.

Sequence Specificity

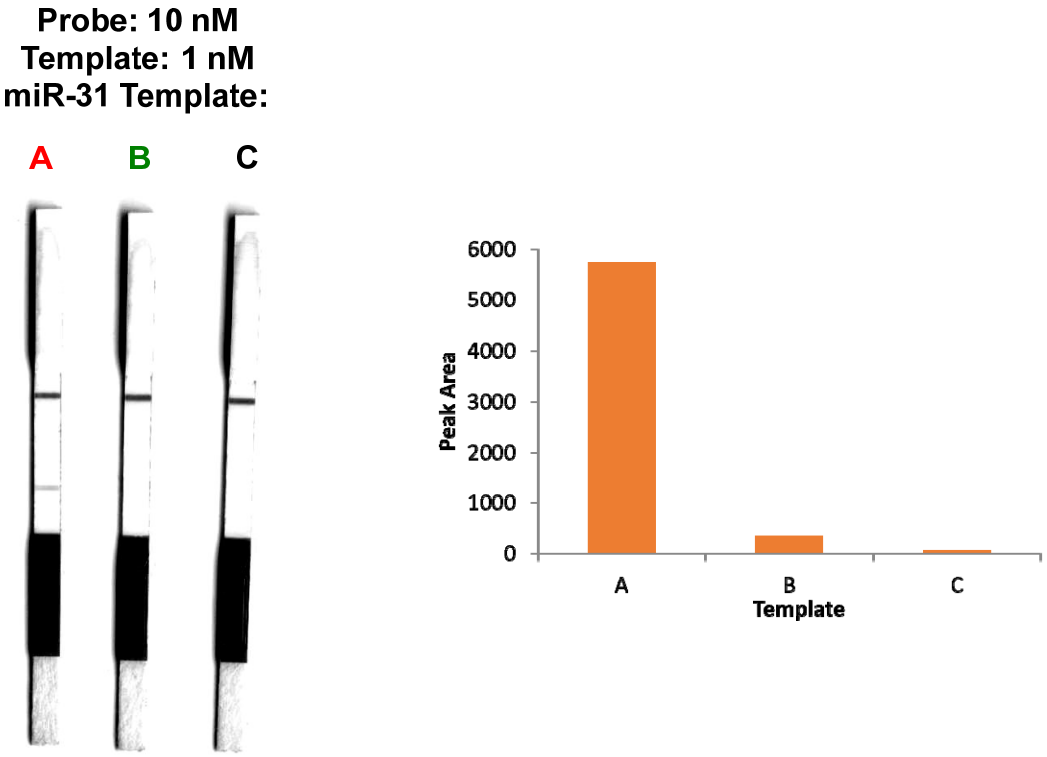
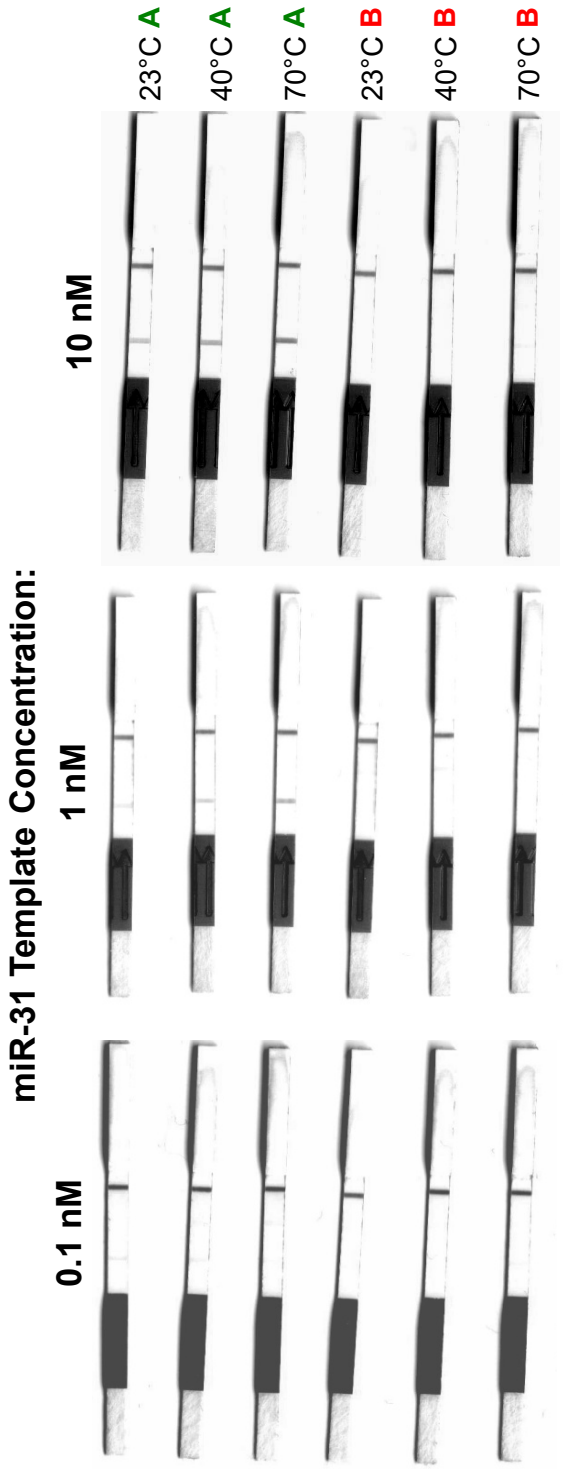


Figure S35. Assay carried out with 10 nM Probe (**11** and **12**) in the presence of 10 nM Template **A**, **B** or **C** at 40 °C; Template **A** (Match): **AGG CAA GAT GCT GGC ATA GCT;** Template **B** (Partial Mismatch): **AGG AAT GAT GCT GGC ATA GCT;** Template **C** (Mismatch): **TAG CTT ATC AGA CTG ATG.** Digital photograph taken of test strip and image processed using ImageJ, resulting peak areas of test zone band represented graphically.

Temperature Dependance and Sequence Specificity

Table S1. Assay carried out with 10 nM **11** and **12** probes at varied temperature and concentration of miR-31 Template A and miR-31 Template B; Template **A** (Match): **AGG CAA GAT GCT GGC ATA GCT;** Template **B** (Partial Mismatch): **AGG AAT GAT GCT GGC ATA GCT**



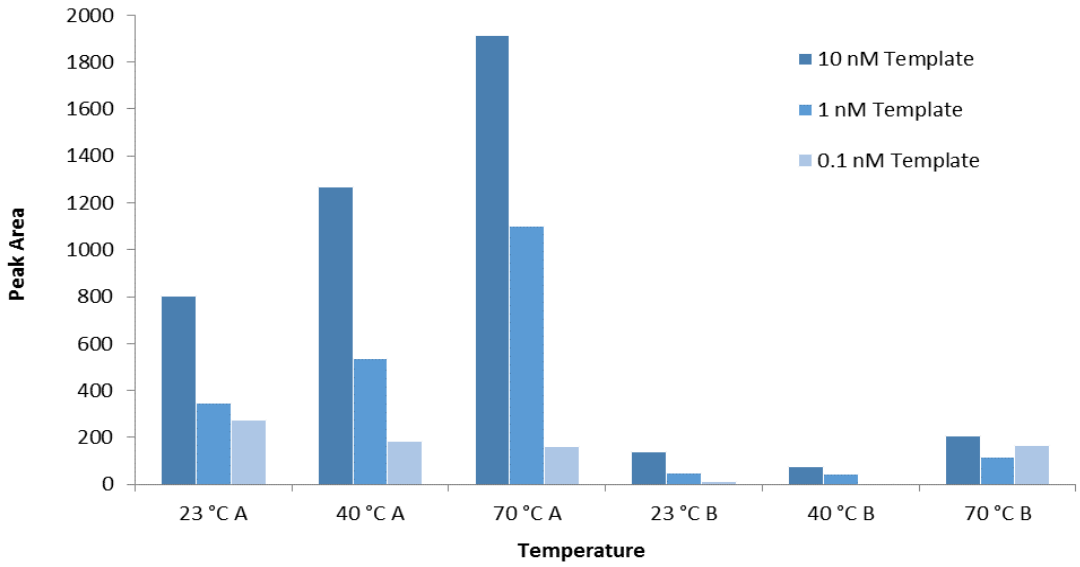


Figure S36. Integrated peak areas (ImageJ) of test strips in Table S1 of assay at varied temperature and template concentration. Template **A** (Match): **AGG CAA GAT GCT GGC ATA GCT**, Template **B** (Partial Mismatch): **AGG AAT GAT GCT GGC ATA GCT.**

Elevated Temperature

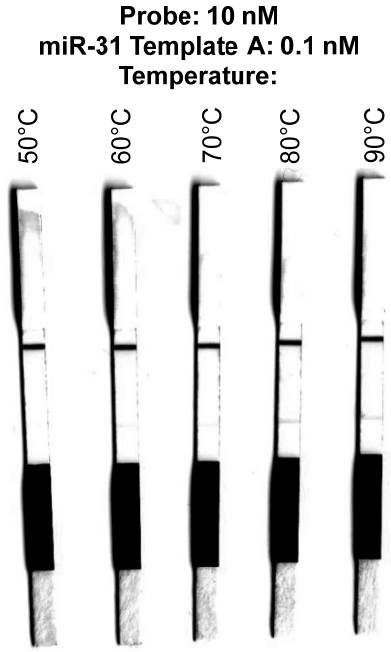


Figure S37. Test Strip results of reaction between probe **11** and **12** in the presence of 0.1 nM miR-31 template A with incubation at specified temperature for 5 minutes.

Selenoester Hydrolysis Rate

23 °C :

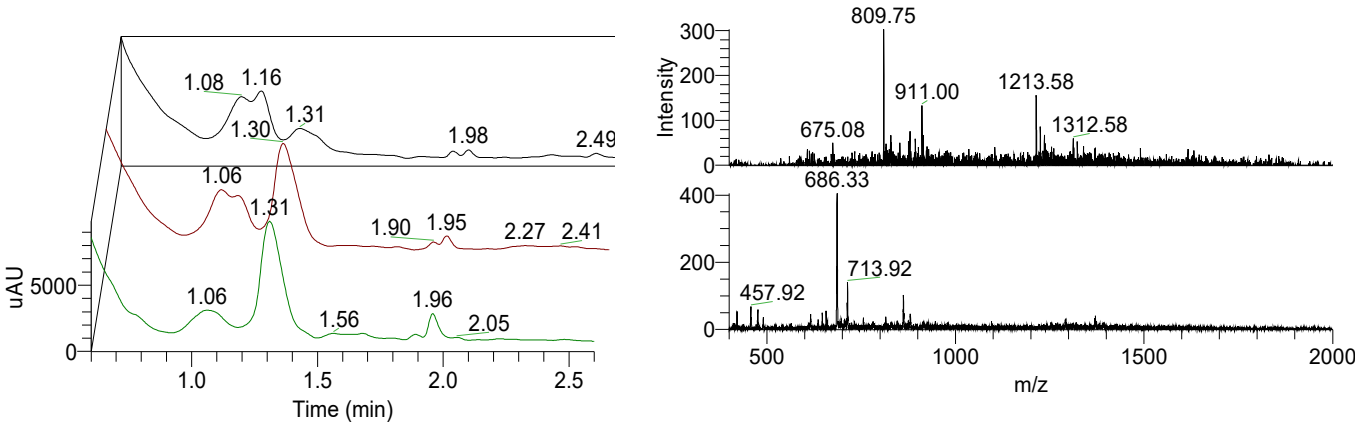


Figure S38. Crude LCMS trace showing hydrolysis of Selenoester **12** at pH 7.0 at room temperature for 5 minutes, 1 h, 2 h.

40 °C :

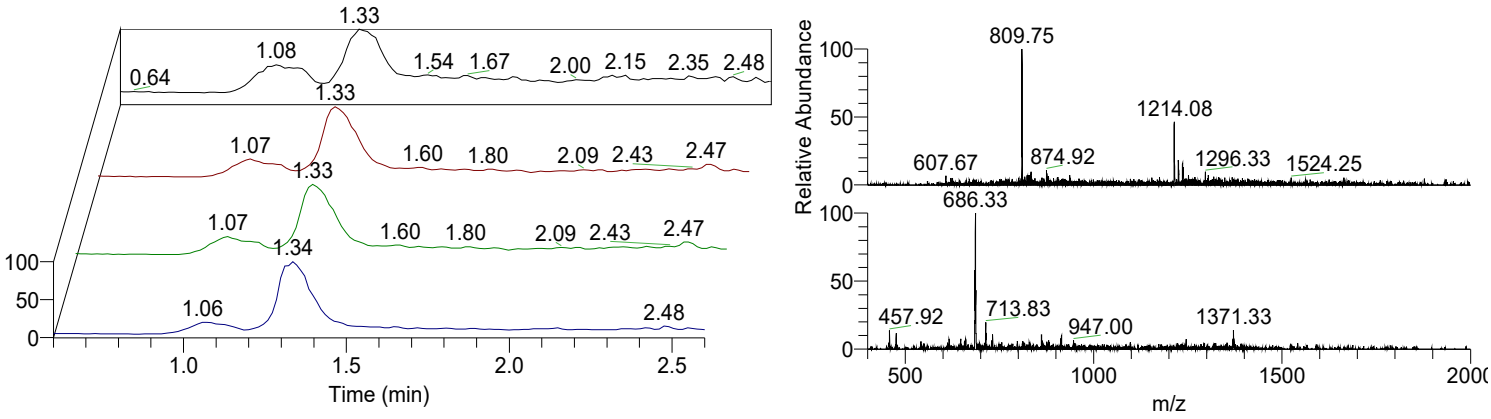


Figure S39. Crude LCMS trace showing hydrolysis of Selenoester **12** at pH 7.0 at 40 °C for 5, 10, 30, 60 minutes

70 °C :

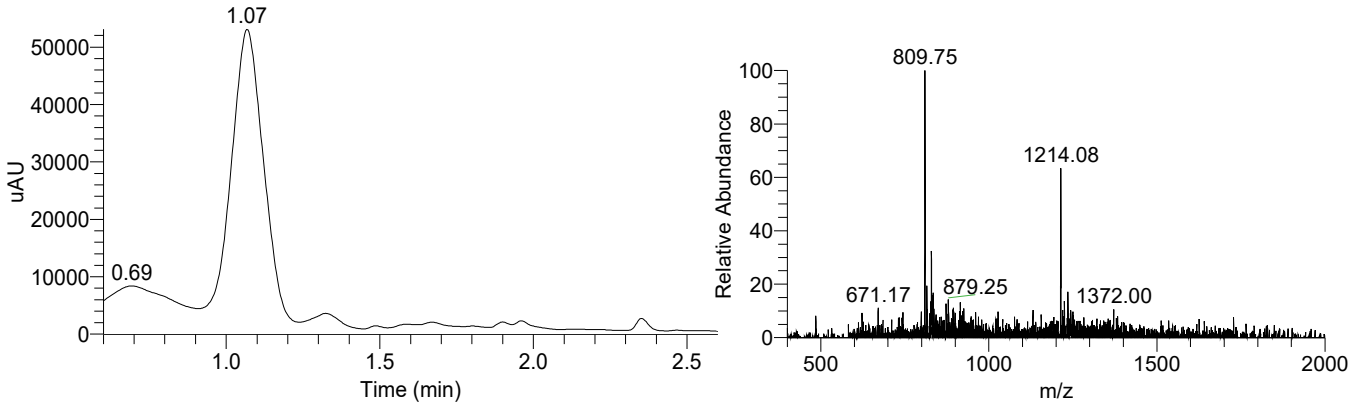


Figure S40. Crude LCMS trace showing hydrolysis of Selenoester **12** at pH 7.0 after incubation at 70 °C for 5 minutes

miRNA Detection in Cell Lysate

HeLa (cervix cancer cell line), MCF-7 (breast cancer cell line) and HEK293T (immortalized human embryonic kidney cell line) cells were cultured in DMEM supplemented with 10% FBS and 1% PenStrep at 37 °C in humidified atmosphere containing 5% CO_2. Cells were lysed by shearing through a 25 G needle in PBS buffer followed by centrifugation at 15,000 rpm for 12 minutes at 5 °C. Aliquots of 100 µL lysate were stored at -20 °C in 1.5 mL eppendorf tubes.

To perform the miRNA detection assay, a 100 µL aliquot of the desired cell lysate was thawed to room temperature. A solution of TCEP in PBS buffer (100 µM, pH 7.0), was added to the eppendorf containing the lysate to give a final TCEP concentration of 5 µM. The eppendorf was then warmed to 40 °C for 5 minutes. The diselenide probe (miR-31: **11**, miR-21: **S1**) closely followed by the selenoester probe (miR-31: **12**, miR-21: **S2**) were added from 1 µM stock solutions in DMF to a final concentration of 10 nM. A stopwatch was started immediately upon addition of the selenoester probe and the reaction was allowed to proceed for 5 minutes at 40 °C before the insertion of a Milenia® HybriDetect 2T test strip. The solution was allowed to migrate by capillary flow through the test strip which was then removed and left to dry at room temperature. The results were evaluated visually.

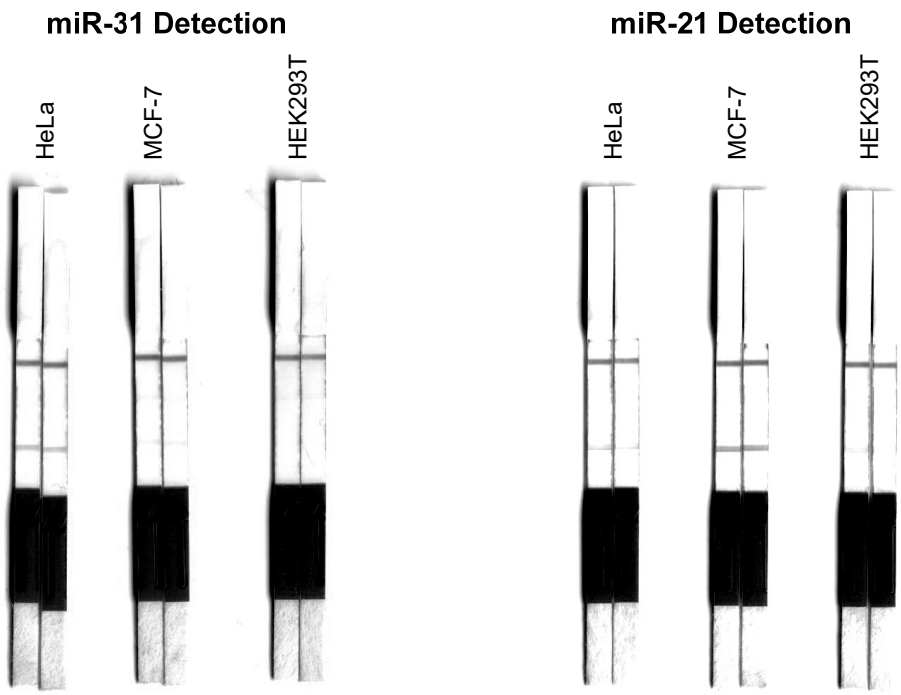


Figure S41. Milenia® HybriDetect 2T test strip results for **A.** miR-31 detection by probes **11** and **12** and **B.** miR-21 detection by probes **S1** and **S2** in HeLa, MCF-7 and HEK293T cell lysate.

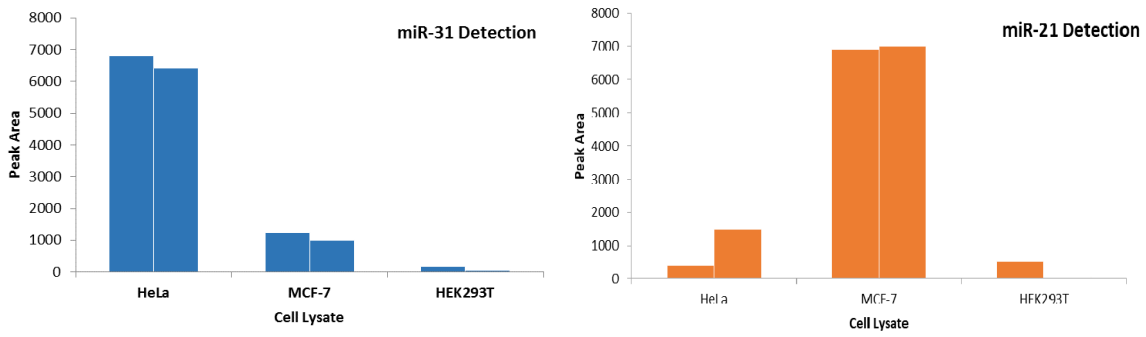


Figure S42. Integrated peak areas of test strip results for miR-31 and miR-21 detection in HeLa, MCF-7 and HEK293T cell lysates.