

## Supplemental Information

### Stimuli-Responsive Assembly of Bilingual Peptide Nucleic Acids

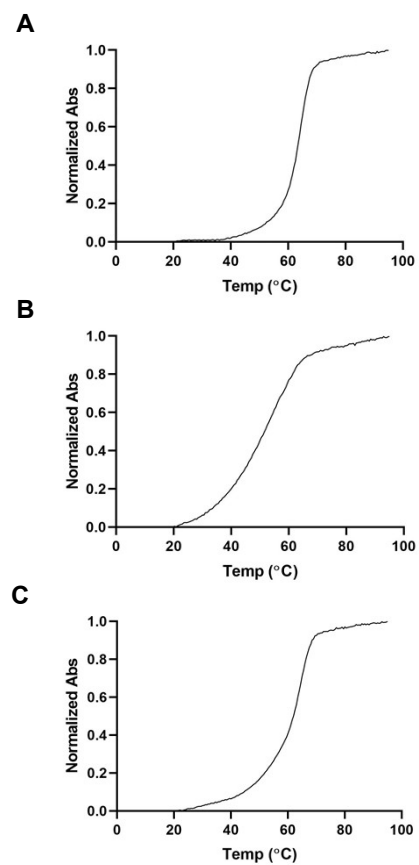
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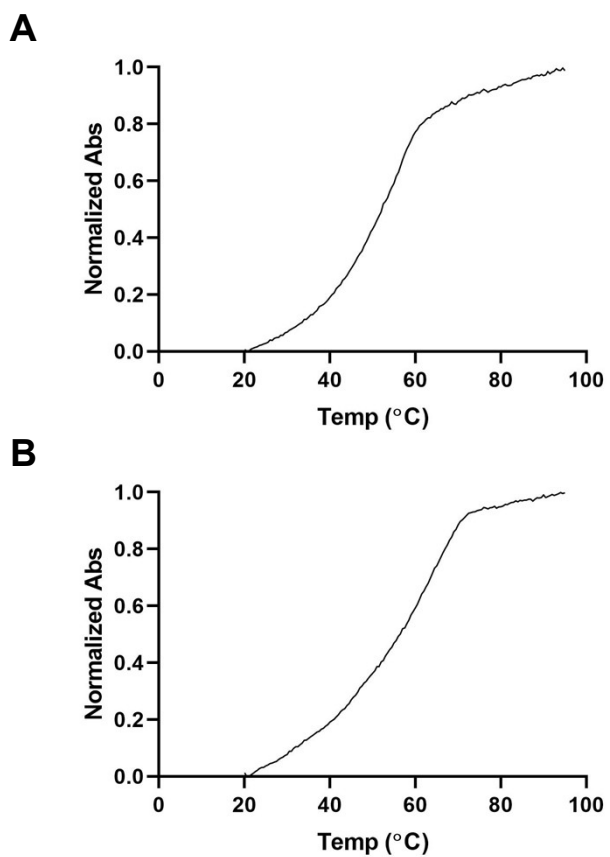
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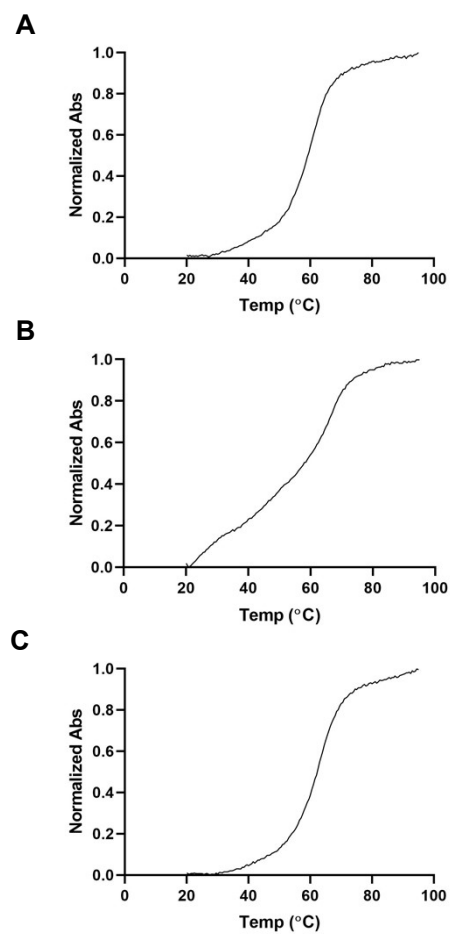
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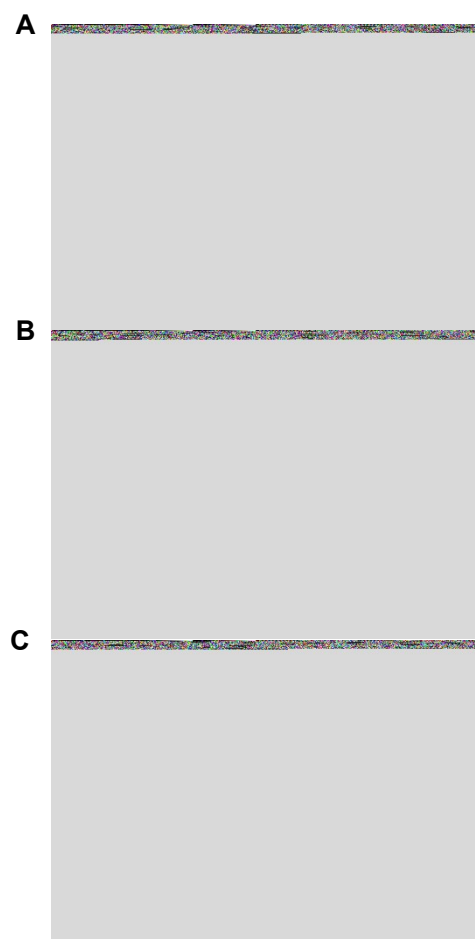
**Fig S1** UV-Vis melting curves using normalized absorbance. Samples were prepared at  $3\mu\text{M}$  in 1X PBS. (A) MS-D:RS-D. (B) MS-D:RS-R. (C) MS-D-m1:RS-D-m1.



**Fig S2** UV-Vis melting curves using normalized absorbance. Sample were prepared at  $3\mu\text{M}$  in 1X PBS. (A) MS-R:RS-D. (B) MS-R:RS-R.



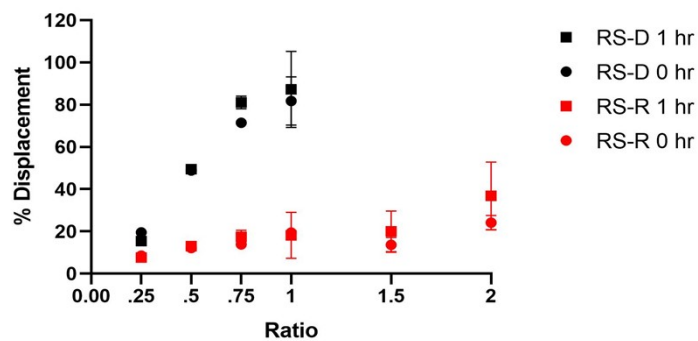
**Fig S3** UV-Vis melting curves using normalized absorbance. Sample were prepared at 3 $\mu$ M in 1X PBS. (A) PNA-C1-FAM:MS-D. (B) PNA-C1-FAM:MS-R. (C) PNA-C1-FAM:MS-D-m1.



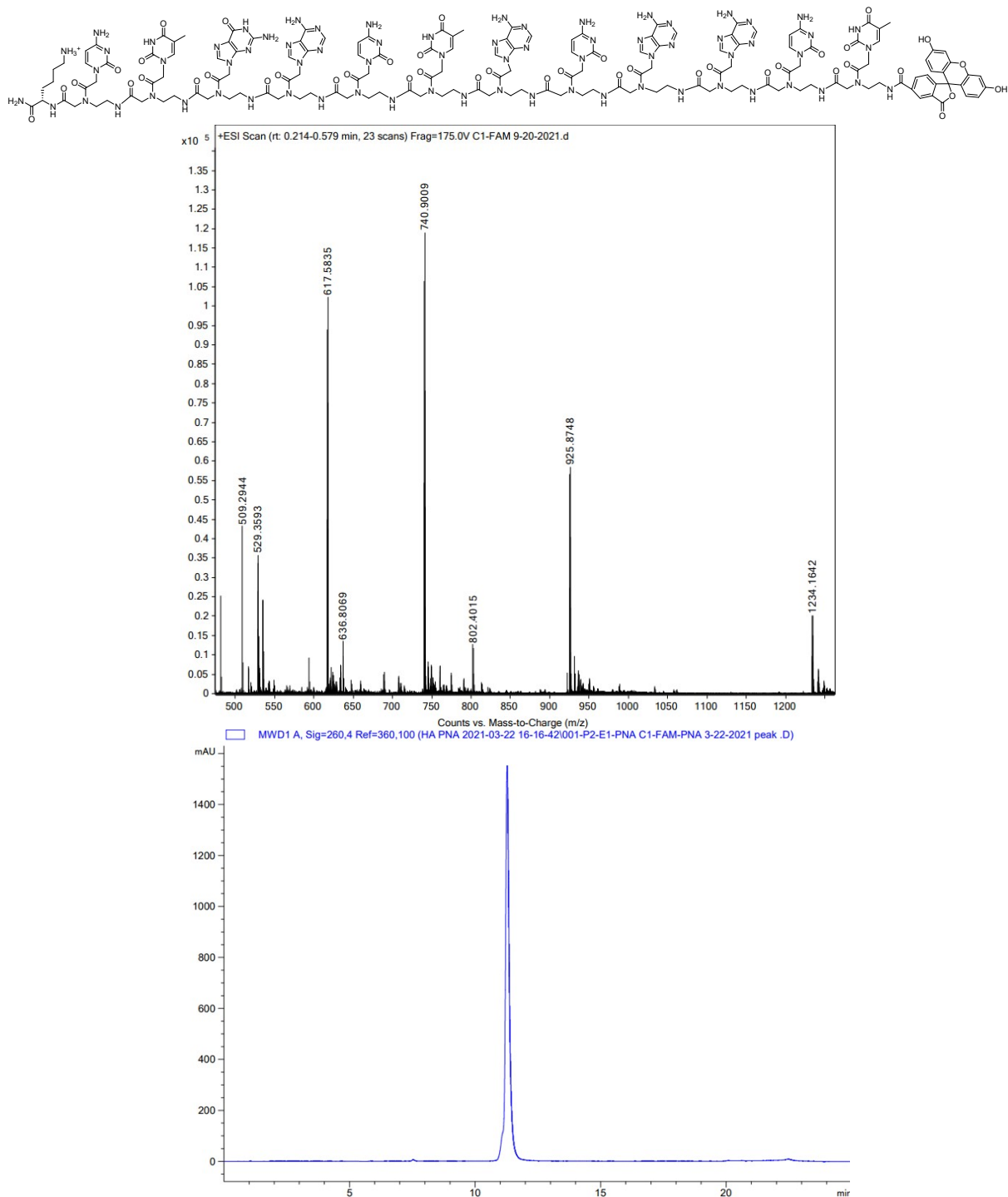
**Fig S4** UV-Vis melting curves using normalized absorbance. Sample were prepared at 3 $\mu$ M in 1X PBS. (A) PNA-A1-FAM:MS-D. (B) PNA-A1-FAM:MS-R. (C) PNA-A1-FAM:MS-D-m1

**Table S1** Melting temperature measurements using normalized absorbance

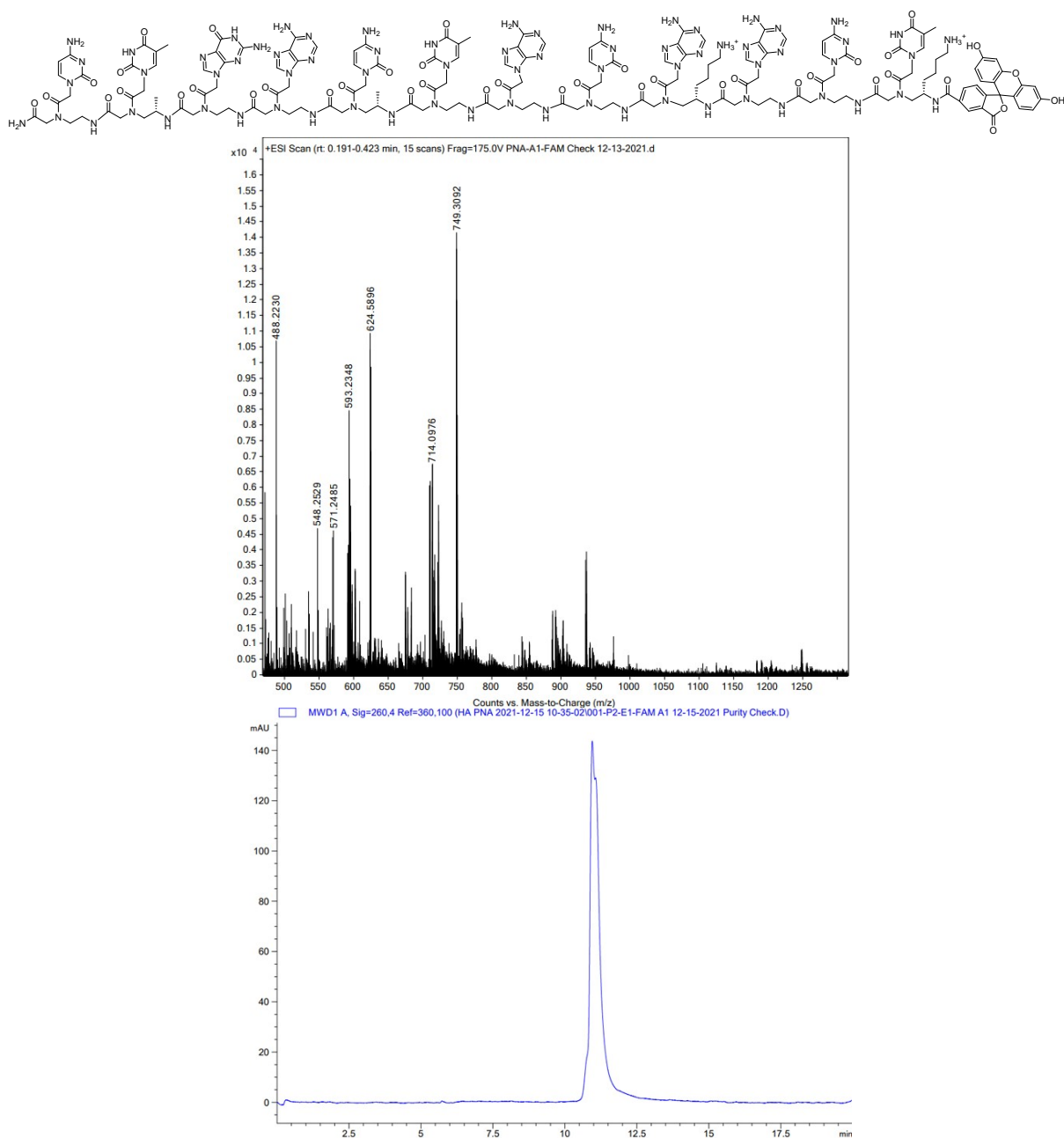
Sequences	Melt Temp (°C)
MS-D:RS-D	62.9 ± 0.2
MS-D:RS-R	50.8 ± 0.2
MS-D-m1:RS-D-m1	61.4 ± 1.3
MS-R:RS-D	51.3 ± 0.5
MS-R:RS-R	56.1 ± 1.2
PNA-C1-FAM:MS-D	58.9 ± 0.1
PNA-C1-FAM:MS-R	58.3 ± 0.6
PNA-C1-FAM:MS-D-m1	61.8 ± 0.2
PNA-A1-FAM:MS-D	56.6 ± 1.1
PNA-A1-FAM:MS-R	54.9 ± 1.1
PNA-A1-FAM:MS-D-m1	53.4 ± 0.8



**Fig S5** % Displacement from PNA-C1-FAM:MS-D using RS-D and RS-R, respectively, was evaluated in relation to the stoichiometry of the system. PNA:MS-D system was used at 3  $\mu$ M in 1xPBS. RS-R was tested up to a stoichiometry of 1:2 duplex:RS-R. Error bars represent standard error (n=3).



**Fig S6** The chemical structure of PNA-C1-FAM. The mass of the sequence was confirmed using ESI-TOF mass spectrometry and purified using RP-HPLC.



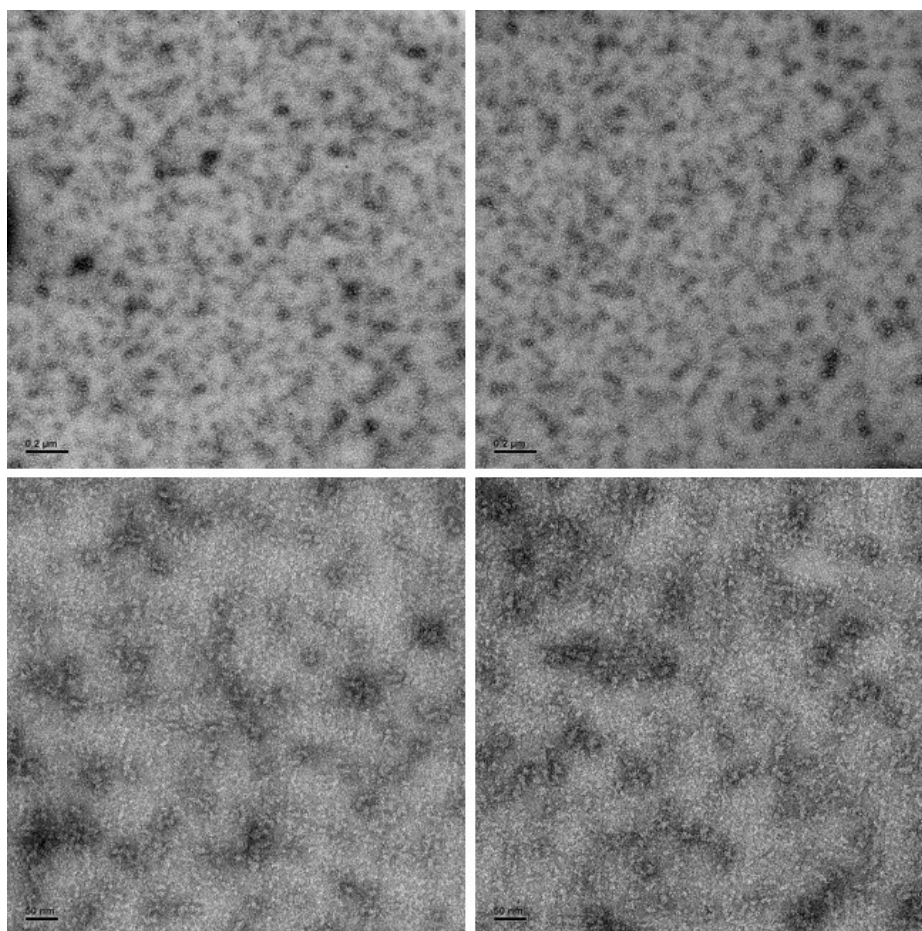
**Fig S7** The chemical structure of PNA-A1-FAM. The mass of the sequence was confirmed using ESI-TOF mass spectrometry and purified using RP-HPLC.



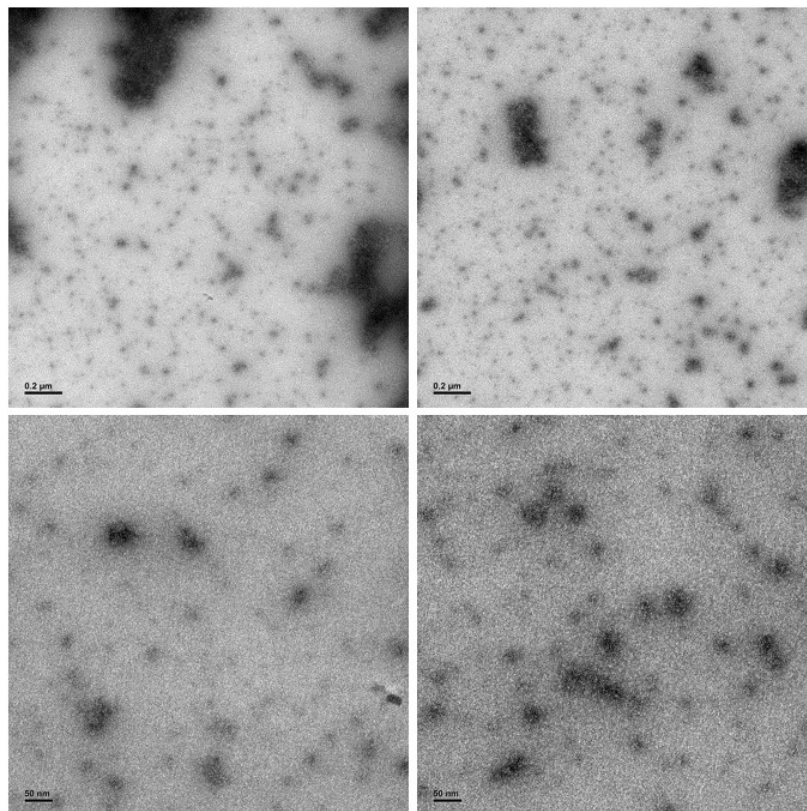
**Table S2** PNA Sequence, expected mass, and found mass

Strand	Sequence	Expected Mass (M+5) <sup>5+</sup>	Found Mass (M+5) <sup>5+</sup>
PNA-C1-FAM	C-C T G A C T A C A A C T FAM-N	740.3720	740.9009
PNA-A1-FAM	C-C T <sub>A</sub> G A C <sub>A</sub> T A C <sub>A<sub>K</sub></sub> A C T <sub>K</sub> FAM-N	748.9794	749.3092

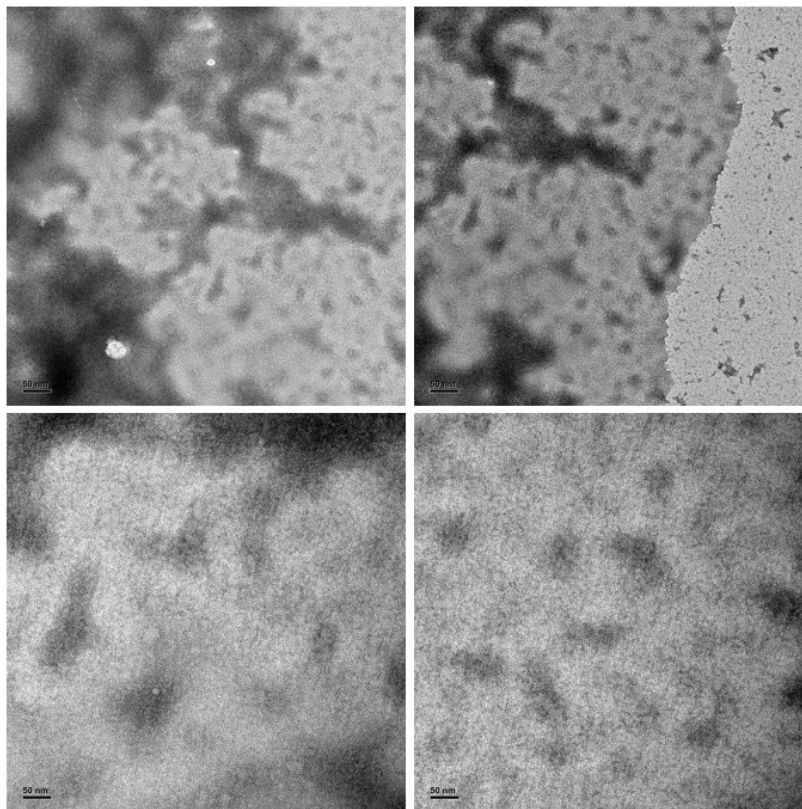
“FAM” denotes 5-carboxyfluorescein. Subscripts denote the amino acid residues incorporated at the  $\gamma$ -position. Masses were confirmed by ESI-TOF mass spectrometry.



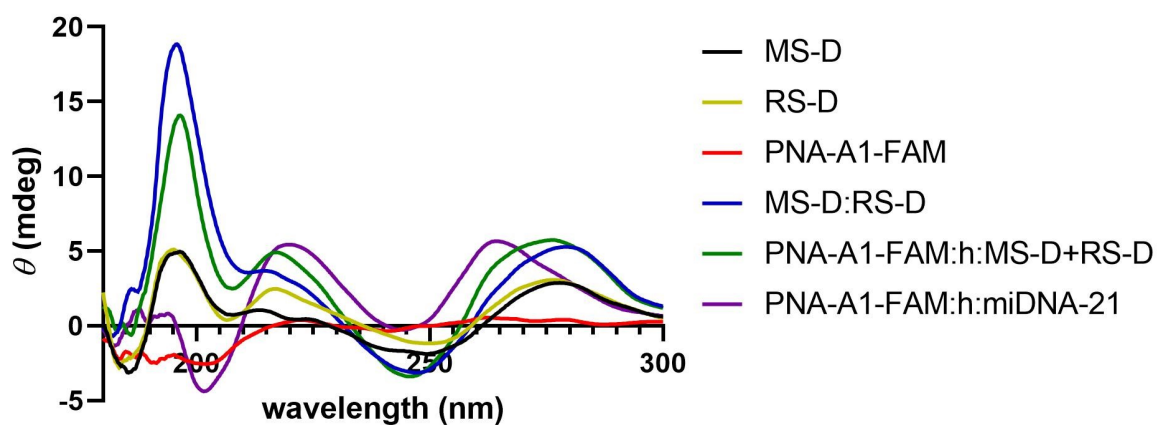
**Fig S8** TEM images of PNA-A1-FAM 100  $\mu$ M in 1X PBS. Top row scale bar = 2000 nm. Bottom row scale bar = 50 nm.



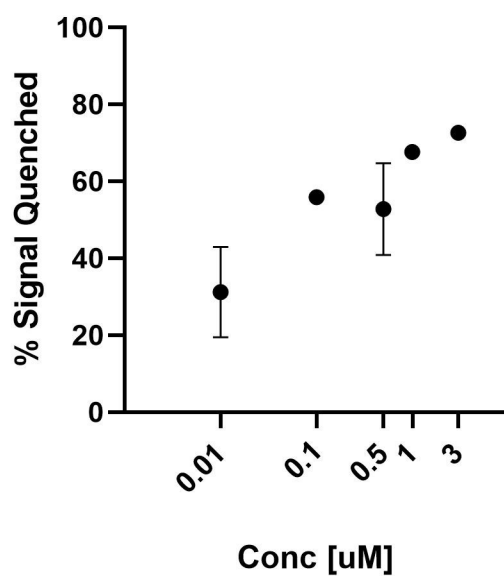
**Fig S9** TEM images of PNA-A1-FAM + MS-D 100  $\mu\text{M}$  in 1X PBS. Top row scale bar = 2000 nm. Bottom row scale bar = 50 nm.



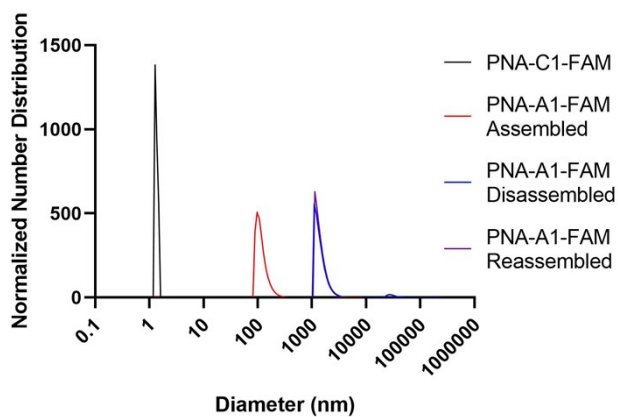
**Fig S10** TEM images of PNA-A1-FAM + MS-D + RS-D 100  $\mu$ M in 1X PBS. Top row scale bar = 2000 nm. Bottom row scale bar = 50 nm.



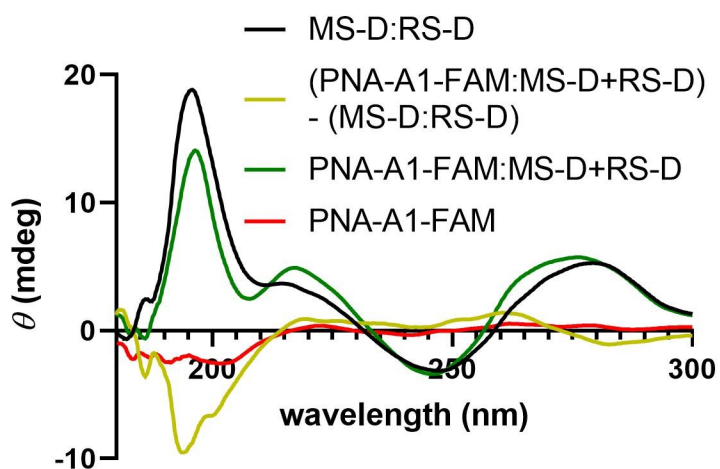
**Fig S11** CD spectroscopy demonstrating the change in maxima and minima upon the addition of RS-D-0. All samples were prepared using 100  $\mu$ M PNA and DNA in 1x PBS.



**Fig S12** Hybridization of MS-D to PNA-A1-FAM was monitored using fluorescence quenching. Error bars represent standard error (n=3).



**Fig S13** Normalized size distribution of PNA assemblies. Samples tested at 200 µM in 1x PBS. Average diameter of particles of PNA-C1-FAM =  $1.5 \pm 0.3$  nm and  $80.4 \pm 22.8$  nm; PNA-A1-FAM (assembled) =  $119.1 \pm 34.0$  nm. PNA-A1-FAM (disassembled) =  $1438.9 \pm 400.2$  nm. PNA-A1-FAM (reassembled) =  $1470.5 \pm 417.2$  nm.



**Fig S14** CD spectroscopy of MS-D+RS-D signal subtracted by PNA-A1-FAM:MS-D+RS-D signal to achieve PNA-A1-FAM signal. All samples were prepared using 100  $\mu$ M PNA and DNA in 1x PBS.