

RNA Secondary Structure-Based Design of Antisense Peptide Nucleic Acids for Modulating Disease-Associated Aberrant Tau pre-mRNA Alternative Splicing

Alan Ann Lerk Ong ^{1,2,†}, **Jiazi Tan** ^{2,†}, **Malini Bhadra** ³, **Clément Dezanet** ⁴, **Kiran M. Patil** ², **Mei Sian Chong** ⁵, **Ryszard Kierzek** ⁶, **Jean-Luc Decout** ⁴, **Xavier Roca** ³ and **Gang Chen** ^{2,*}

¹ NTU Institute for Health Technologies (HeathTech NTU), Interdisciplinary Graduate School, Nanyang Technological University, 50 Nanyang Drive, Singapore 637553, Singapore

² Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371, Singapore

³ School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore

⁴ University Grenoble Alpes/CNRS, Département de Pharmacochimie Moléculaire, ICMG FR 2607, UMR 5063, 470 Rue de la Chimie, F-38041 Grenoble, France

⁵ Geriatric Education & Research Institute, 2 Yishun Central 2, Singapore 768024, Singapore

⁶ Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland

* Correspondence: RNACHEN@ntu.edu.sg; Tel: +65 6592 2549; Fax: +65 6791 1961

† These authors contributed equally to this work.

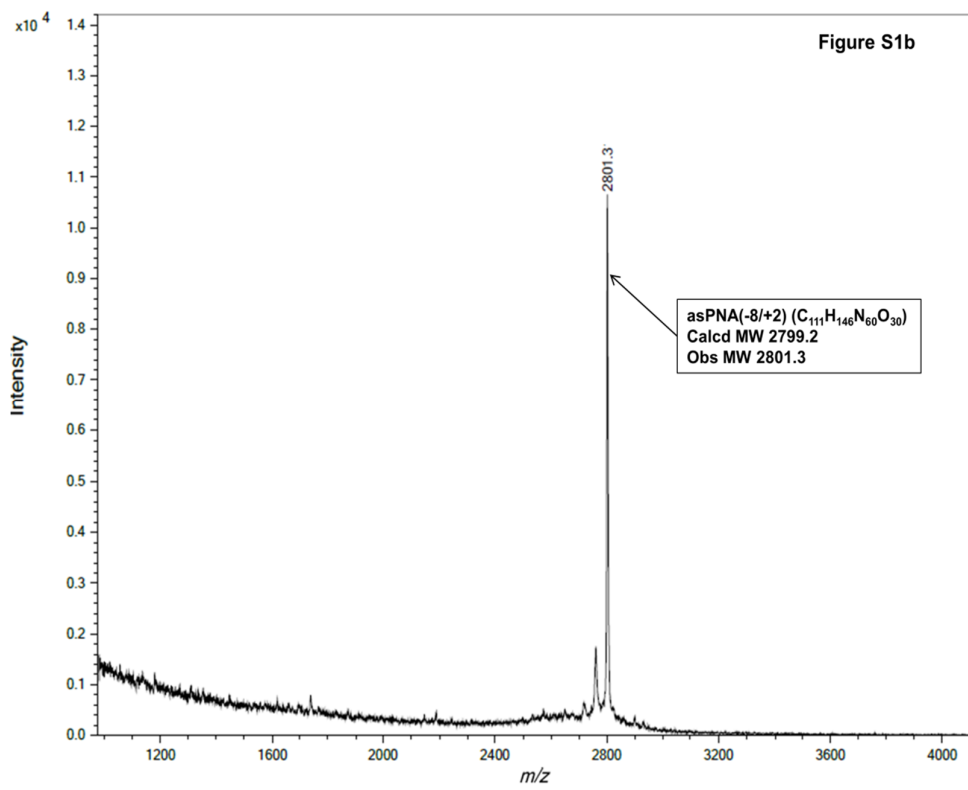
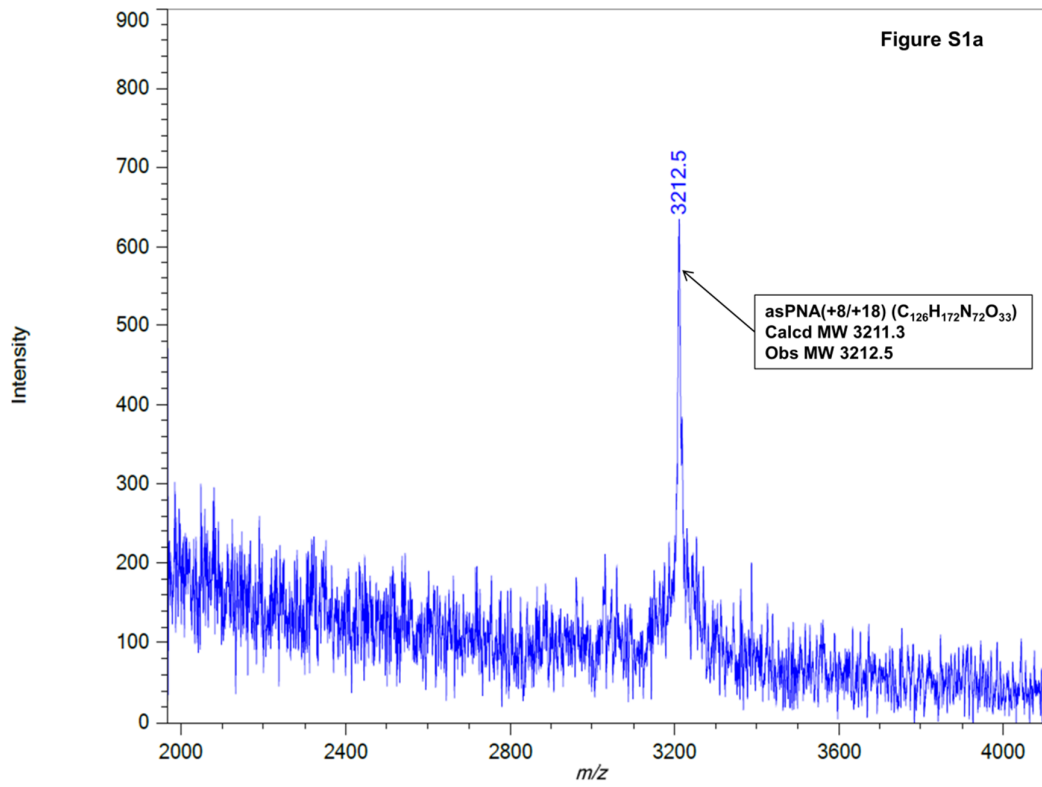
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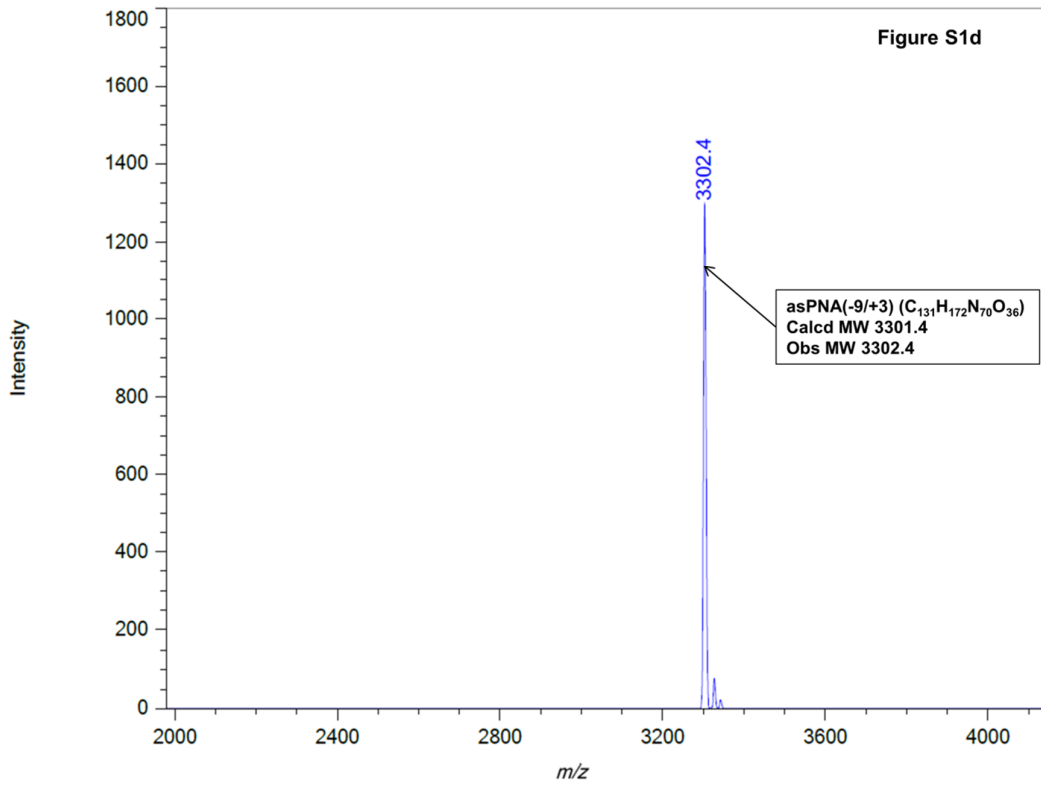
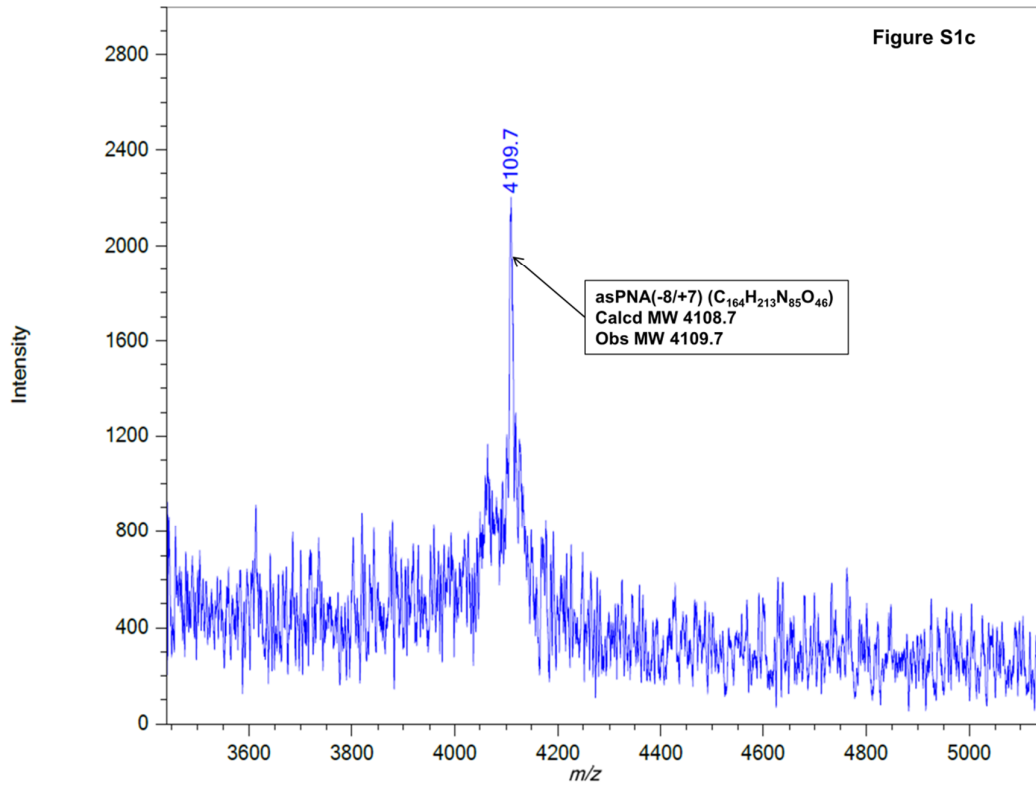
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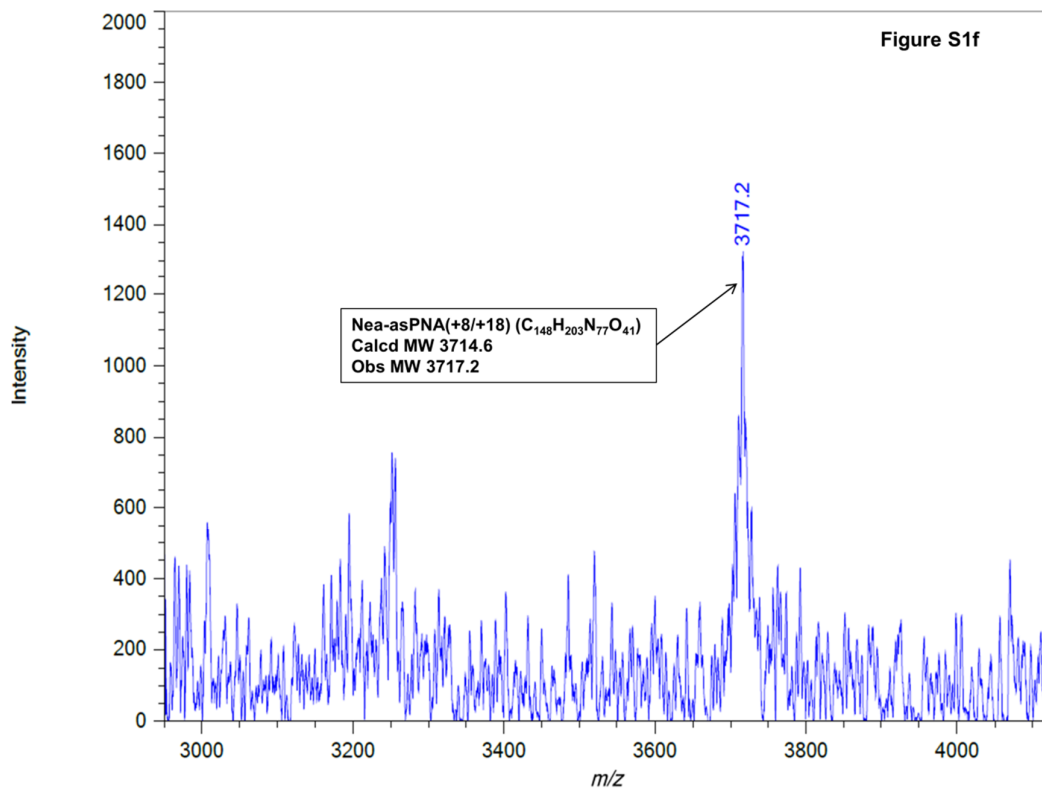
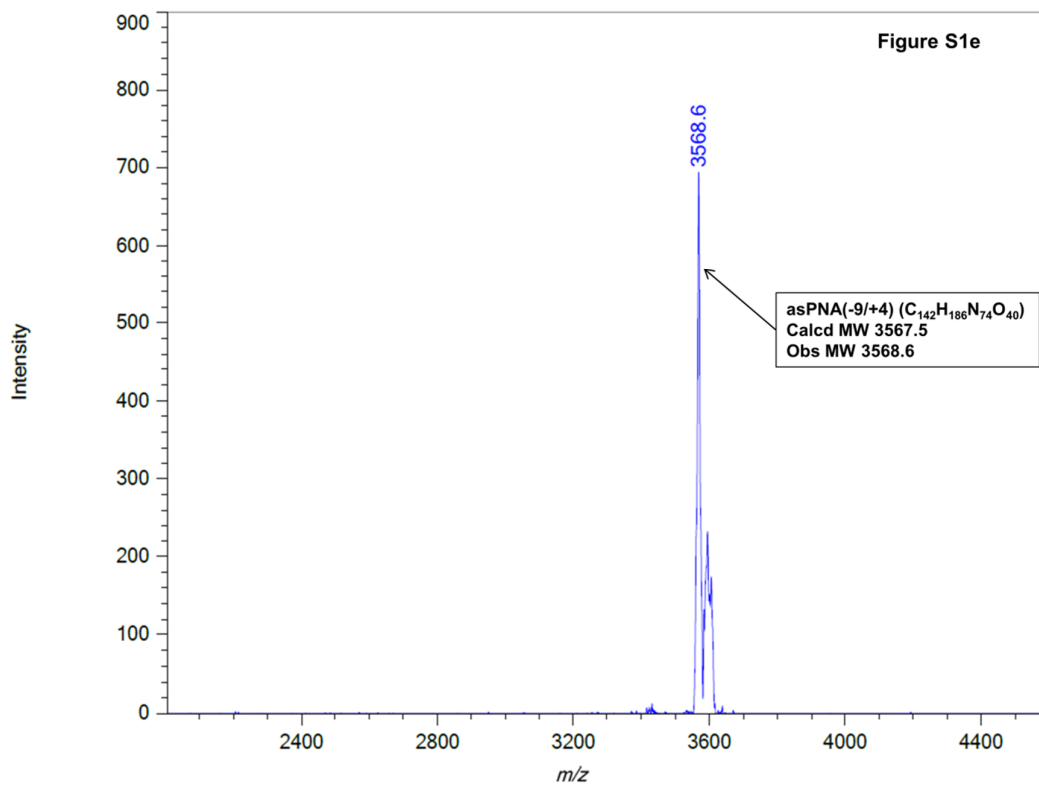
2. MALDI-TOF of PNA oligomers

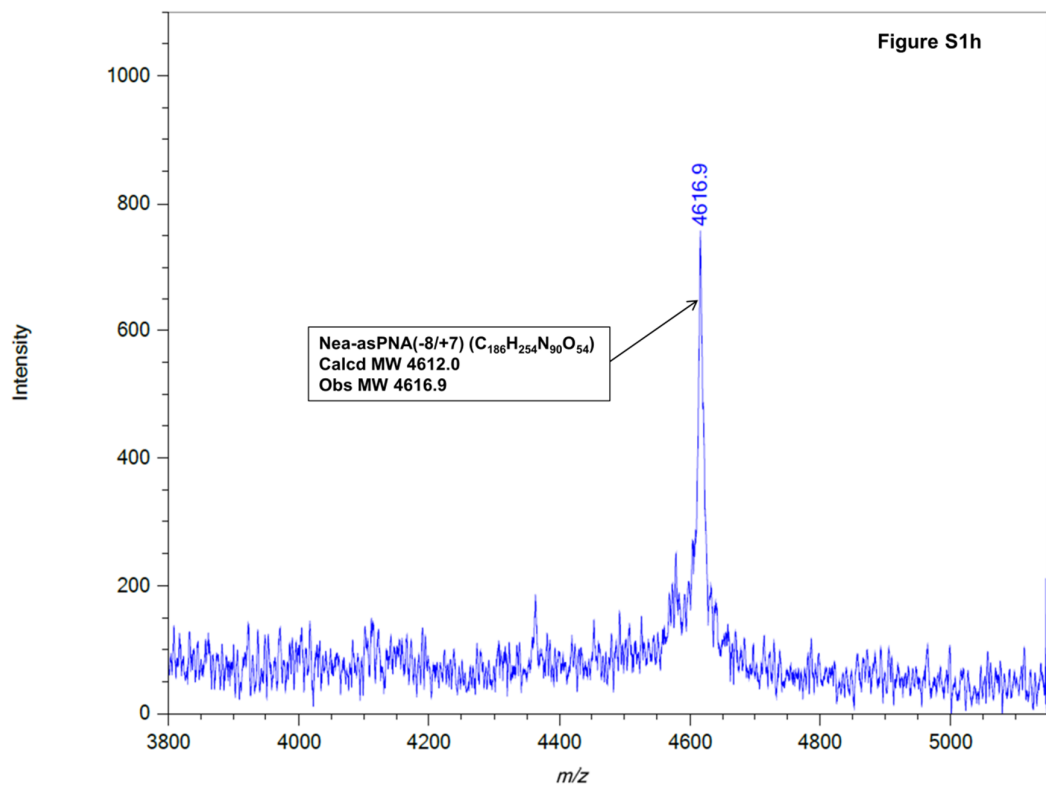
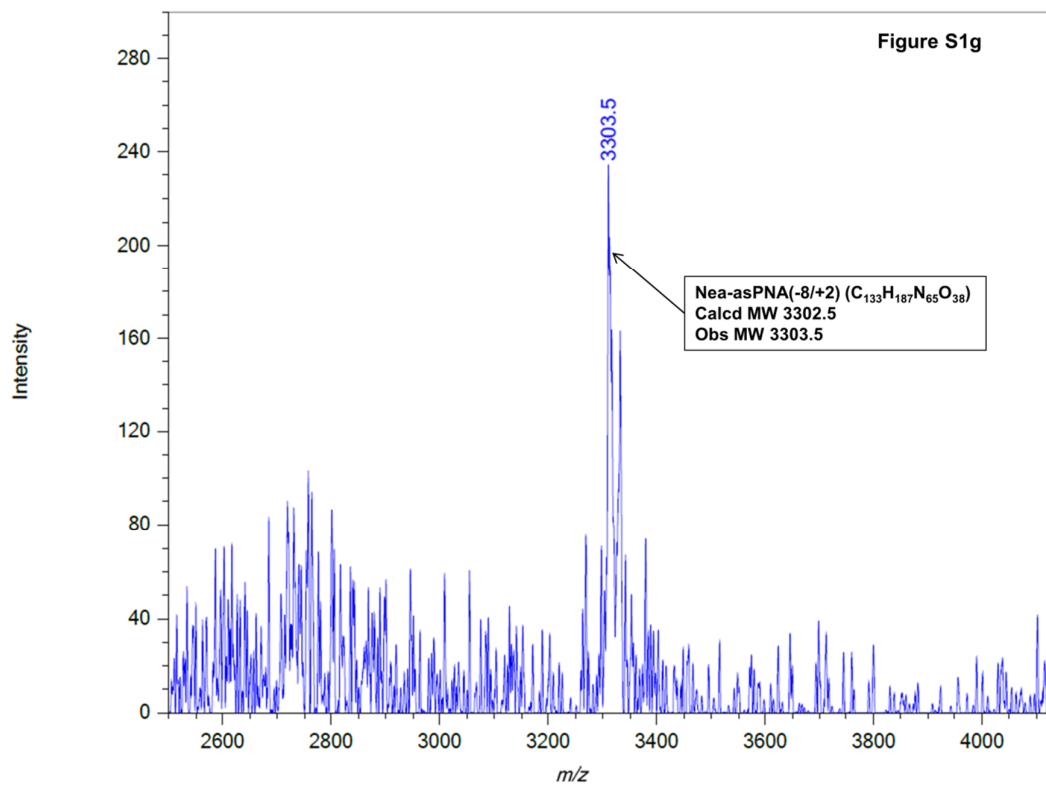
Table S1. Sequences and MALDI-TOF data for synthesized PNAs and PNA-neamine conjugates.

PNA	Sequence	Molecular Formula	Calculated MW	Observed MW
asPNA(+8/+18)	NH ₂ -Lys-ACGTGTGAAGG-CONH ₂	C ₁₂₆ H ₁₇₂ N ₇₂ O ₃₃	3211.3	3212.5
asPNA(-8/+2)	NH ₂ -Lys-ACACTGCCGC-CONH ₂	C ₁₁₁ H ₁₄₆ N ₆₀ O ₃₀	2799.2	2801.3
asPNA(-8/+7)	NH ₂ -Lys-TACTCACA CTGCCGC-CONH ₂	C ₁₆₄ H ₂₁₃ N ₈₅ O ₄₆	4108.7	4109.7
asPNA(-9/+3)	NH ₂ -Lys-CACA CTGCCGCC-CONH ₂	C ₁₃₁ H ₁₇₂ N ₇₀ O ₃₆	3301.4	3302.4
asPNA(-9/+4)	NH ₂ -Lys-TCACA CTGCCGCC-CONH ₂	C ₁₄₂ H ₁₈₆ N ₇₄ O ₄₀	3567.5	3568.6
asPNA(+8/+18)-Nea	NH ₂ -Nea-Lys-ACGTGTGAAGG-CONH ₂	C ₁₄₈ H ₂₀₃ N ₇₇ O ₄₁	3714.6	3717.2
asPNA(-8/+2)-Nea	NH ₂ -Nea-Lys-ACACTGCCGC-CONH ₂	C ₁₃₃ H ₁₈₇ N ₆₅ O ₃₈	3302.5	3303.5
asPNA(-8/+7)-Nea	NH ₂ -Nea-Lys-TACTCACA CTGCCGC-CONH ₂	C ₁₈₆ H ₂₅₄ N ₉₀ O ₅₄	4612.0	4616.9
asPNA(-9/+3)-Nea	NH ₂ -Nea-Lys-CACA CTGCCGCC-CONH ₂	C ₁₅₃ H ₂₁₃ N ₇₅ O ₄₄	3804.7	3805.7
asPNA(-9/+4)-Nea	NH ₂ -Nea-Lys-TCACA CTGCCGCC-CONH ₂	C ₁₆₄ H ₂₂₇ N ₇₉ O ₄₈	4070.8	4071.9









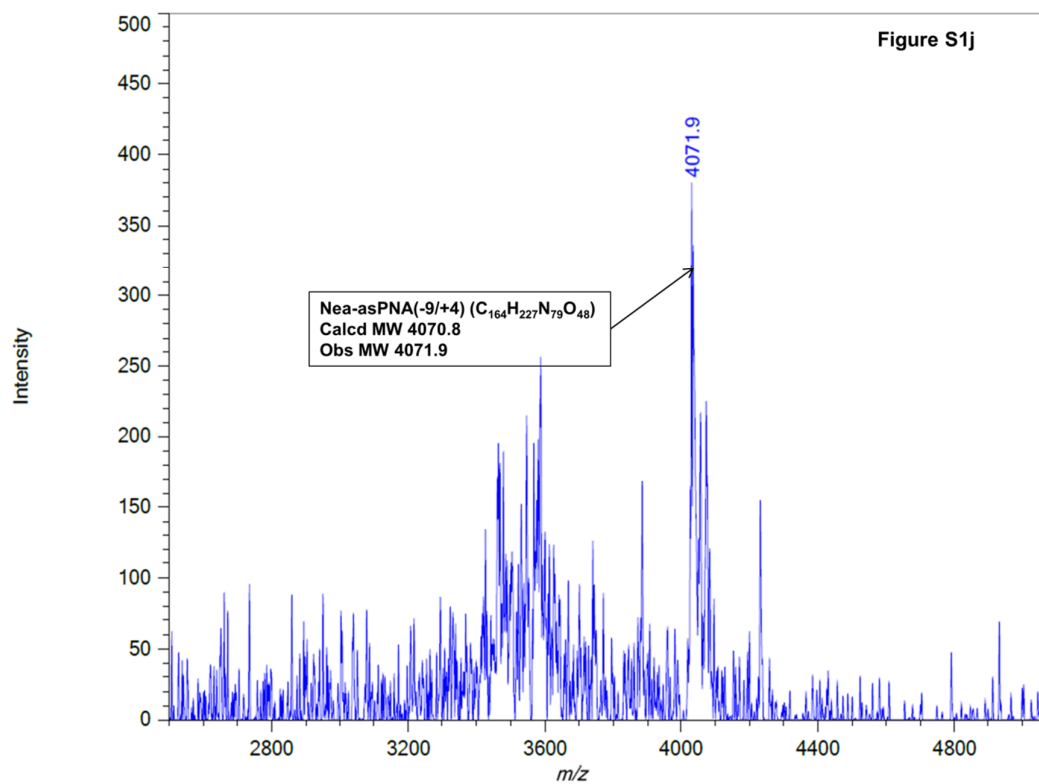
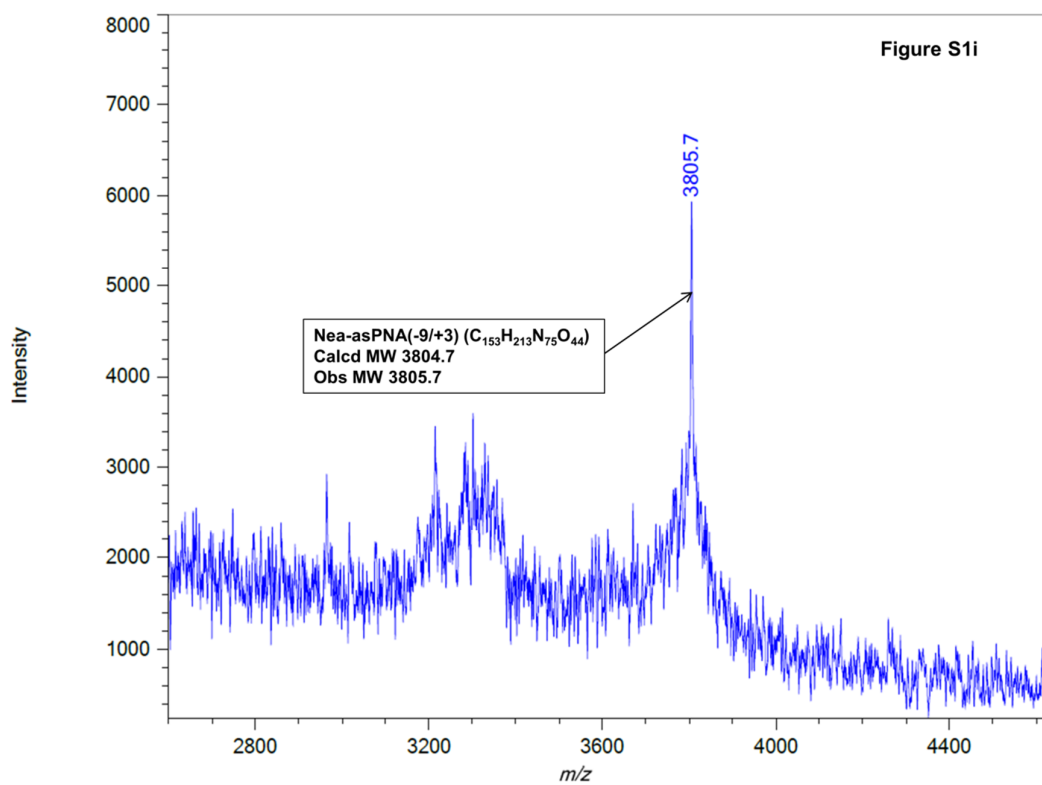


Figure S1. MALDI-TOF data of PNAs and PNA-neamine conjugates studied in this paper.

3. Non-denaturing PAGE results

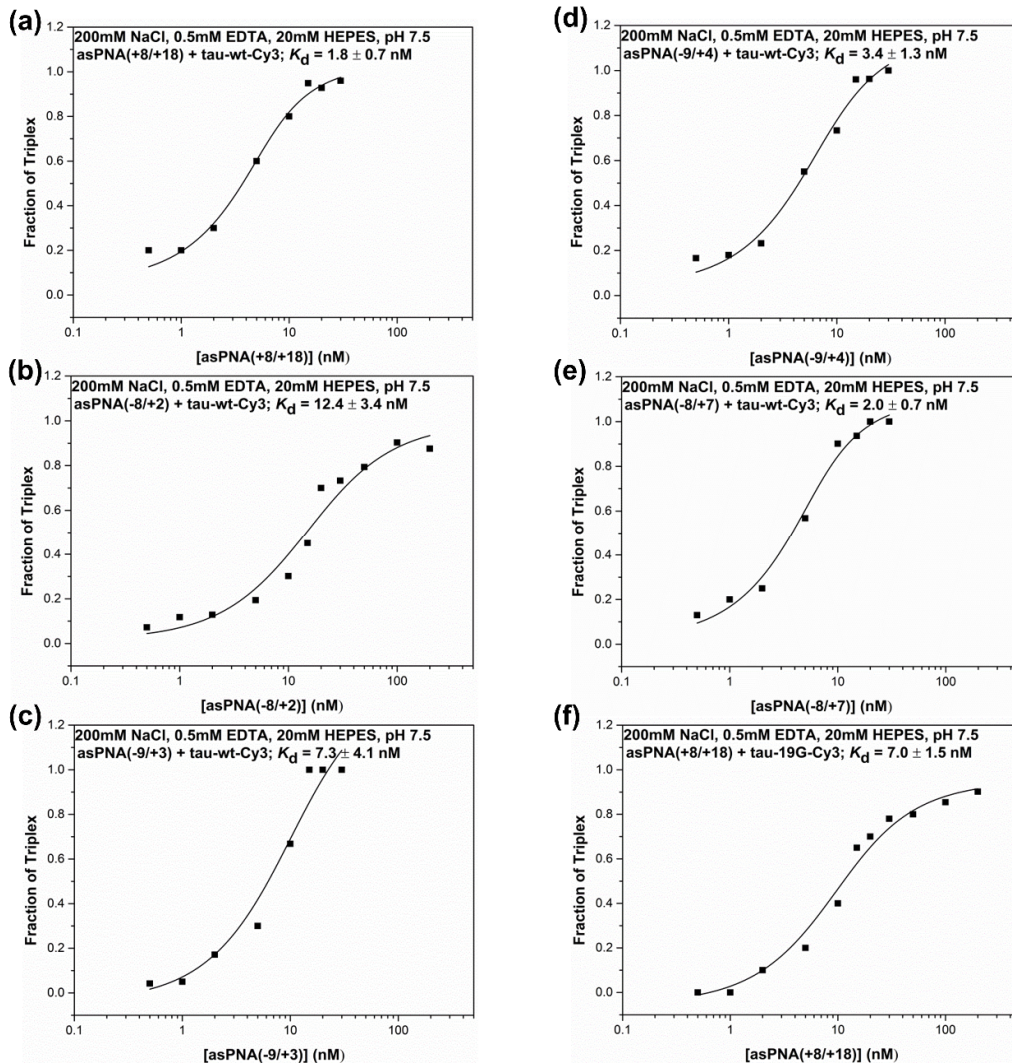


Figure S2. K_d determination by nondenaturing PAGE for various PNAs binding to tau pre-mRNA hairpins. The fraction of RNA-PNA complex formation was calculated based on the band intensities. The data were fit to the equation: $Y = Y_0 + (B/2/R_0)(R_0 + X + K_d - ((R_0 + X + K_d)^2 - 4R_0X)^{1/2})$ where R_0 is the RNA hairpin concentration (5 nM). Y_0 and B are the minimum and maximum fraction of triplex formation respectively. X is the total PNA concentration and K_d is the dissociation constant. For panels a, c-e, the points at the highest concentrations (50, 100 and 200 nM) are removed to avoid over fitting of the high-concentration base lines.

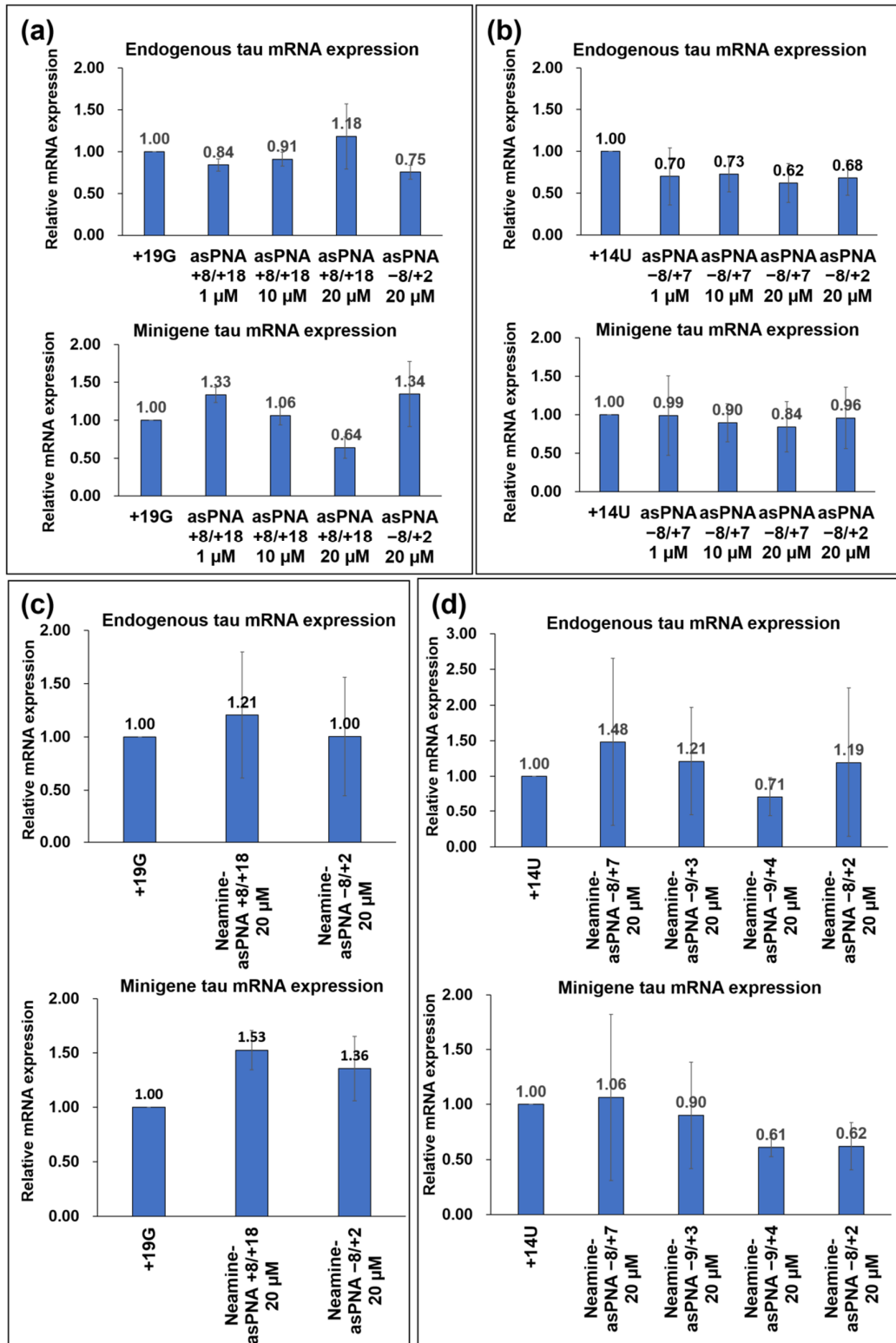


Figure S4: Real-time PCR data showing the transcript levels of endogenous tau and minigenes. (a) Co-transfection of +19G minigene with asPNAs (method A). (b) Co-transfection of minigene +14U and asPNAs (method A). (c) Transfection of minigene +19G, followed by the application of asPNA-neamine conjugates (method B). (d)

Transfection of minigene +14U, followed by the application of asPNA-neamine conjugates (method B). The experiments were performed in triplicates and the data are shown as mean \pm standard deviation. Student's t-test was performed with adjusted P-values using Bonferroni correction [P = 0.0125 for (a), (b), (d); P = 0.025 for (c)].