Supplementary Material

2'FY-RNA aptamers form metastable multimeric G-quadruplexes that selectively bind pyoverdines

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1 Synthesis of pyoverdine chromophores

Amide ester acid 4. To a round bottom was added nitro lactam A followed by ethyl bromoacetate, DMF, and NaH. The solution was heated to 100oC overnight and quenched with aq. sodium bicarbonate. Washes with ethyl acetate were done 3x (10 mL/ 1 mmol) and the organics were dried over sodium sulfate. The solution was filtered over cotton and concentrated in vacuo. The crude material was separated by column chromatography with ethyl acetate and hexane gave the desired nitro lactam ester plus some O-alkylated product. 1H NMR (400 MHz, Chloroform-d) δ 8.64 (s, 1H), 7.08 (s, 1H), 6.53 (s, 1H), 5.15 (s, 2H), 4.25 (q, J = 7.1 Hz, 2H), 4.00 (s, 3H), 3.96 (s, 3H), 1.37 – 1.17 (m, 3H). 13C NMR (101 MHz, Chloroform-d) δ 167.30, 156.29, 154.06, 146.70, 138.11, 136.14, 110.66, 110.42, 96.53, 96.49, 62.19, 56.49, 56.34, 44.76, 14.19. The nitro group was reduced using excess Fe and aqueous HCl in ethanol. The resulting product was acylated with succinic anhydride to provide amide ester acid 4.

Nitro azetidine ester 1. To an oven dried flask was charged nitro lactam A followed by thionyl chloride (20-30 mol. Eq.) and catalytic amounts of DMF. The solution was heated to 98 °C and left to react overnight. Upon cooling, 2-chloro-6,7-dimethoxy-3-nitro quinoline precipitates as a solid. Thionyl chloride was vacuum distilled at 60 °C with two cold traps until dry. The yellow chloro quinoline obtained was used either in its crude state or re-dissolved in DCM and washed with brine. DCM was dried over sodium sulfate and filtered, and the solvent removed in vacuo to give a yellow solid. 1H NMR (400 MHz, Chloroform-d) δ 8.63 (s, 1H), 7.41 (s, 1H), 7.14 (s, 1H), 4.07 (s, 3H), 4.05 (s, 3H).

To an oven dried flask was charged 2-chloro-6,7-dimethoxy-3-nitro quinoline and the azetidine ester (1 mol. Eq.), followed by DMF (3mL / 1 mmol) and TEA (2.1 mol. Eq.). The solution was heated to 92 oC and stirred overnight. The DMF was vacuum distilled off and the crude material was dissolved in DCM and wet loaded onto a column. Separation with a gradient of hexane:ethyl acetate (1:0 – 6:4) afforded compound 1 as a red solid (~80% yield). 1H NMR (400 MHz, Chloroform-d) δ 8.54 (s, 1H), 7.05 (s, 1H), 6.94 (s, 1H), 5.20 (dd, J = 9.5, 5.6 Hz, 1H), 4.42 (td, J = 8.9, 6.3 Hz, 1H), 4.05- 3.98 (m, 1H), 4.01 (s, 3H), 3.96 (s, 3H), 3.73 (s, 3H, O2Me), 2.71 (dtd, J = 10.7, 9.2, 6.3 Hz, 1H), 2.38 (ddt, J = 11.0, 9.0, 5.7 Hz, 1H). 1H NMR (400 MHz, DMSO-d6) δ 8.77 (s, 1H), 7.37 (s, 1H), 6.97 (s, 1H), 5.08 (dd, J = 9.7, 5.9 Hz, 1H), 4.17 (q, J = 8.3 Hz, 1H), 3.99-3.81(m, 1Hi 3.93 (s, 3H), 3.85 (s, 3H), 3.67 (s, 3H), 2.75 – 2.59 (m, 1H), 2.31 (tt, J = 10.7, 5.7 Hz, 1H). 13C NMR (101 MHz, DMSO-d6) δ 172.30, 156.27, 148.78, 148.42, 146.74, 134.46, 131.56, 116.96, 1s07.16, 105.61, 62.03, 56.44, 56.11, 52.41, 50.69, 31.12.

Amino acid 2 and amide acid 3. A flask was charged with 1 followed by hydroiodic acid (~6 mL/ 1 mmol) and allowed to stir until homogenized. The solution was then heated to 66 oC until dry. The beige material was analyzed without purification. 1H NMR (400 MHz, DMSO-d6) δ 11.37 (s, 1H), 9.03 (s, 1H), 7.52 (s, 2H), 7.30 (s, 1H), 7.09 (s, 1H), 4.40 (d, J = 5.8 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.51 (q, J = 6.1 Hz, 1H), 3.26 (dq, J = 40.4, 8.4 Hz, 2H), 1.92 (q, J = 7.3 Hz, 1H). The crude product was dissolved in chloroform and treated with excess acetic anhydride.

2 Aptamer selection

This protocol was designed to isolate aptamers with the characteristics of high affinities and specificities for the ligand and for switching structure on binding the ligand. In addition, the presence of complementary oligonucleotides to the PCR primer sequences during selection limited structure selection to the single-stranded central randomized region. Aptamer selection started with a high molar ratio of pool to PVD-Pf5 (1:1) and a harmonic increase of pool to target PVD-Pf5 in later cycles to increase the selection pressure[1]. Counter selections were performed after rounds 3 and 6 against a mixture of two other siderophores, enterobactin (ENB) and ornibactin (ORB), to eliminate nonspecific binders (Fig. 1A). With the assumption that the oligonucleotide complex with PVD-Pf5 is stoichiometric, this selection protocol resulted in 60% of PVD-Pf5 bound by oligonucleotide by round 9 (Fig. 1C). Analysis of the NextGen sequencing results showed that incremental rounds of selection were characterized by increases in the unique fraction (number of sequences in the pool divided by the pool size) and the enriched species (sequences present in the pool more than once), which is characteristic of a successful selection procedure (Fig. S2).

The results of next generation sequencing for oligonucleotide pools from several rounds, including the final round of each selection provided the data for identifying potential aptamer sequences. Sequence clusters were identified with Aptasuite[2] and, based on the size of the cluster and the rise in enriched species through the rounds, several oligonucleotides were evaluated for binding the PVD-Pf5 chromophore by identifying increases in pyoverdine fluorescence when bound to the oligonucleotide.

3 Supplementary tables

Table S1. Nucleic acid sequences used in this study.

Commented [MN1]: Sharif, Please correct/update this table

Name	Sequence
Oligo 5627	TAATACGACTCACTATAGGGAGACAAGAATAAACGCTC
Oligo 5628	GAGCGTTTATTCTTGTCTCCC
Oligo 5629	GCCTGTTGTGAGCCTCCTGTCGAA
Oligo 5637	GCCTGTTGTGAGCCTCCTGTCGAAGTATGCGATAGGAGCGAATGATATATATCAAGT TACCACACGGATGTACTTGCTTGAGCGTTTATTCTTGTCTCCC
Oligo 5651	GGGAGACAAGAAUAAACGCUCCCAAAGAGCACUCCAUUCGAUGGUCGUUUUUCGC ACAUUCGCUCCGCGAUAGACGUUCGACAGGAGGCUCACAACAGGC
Oligo 5652	GGGAGACAAGAAUAAACGCUCGUUACGAAGAAACUUUCCCGUUAACGUGCCAAAU UCAUUCGCUCCGAAUUGGGCAUUCGACAGGAGGCUCACAACAGGC
Oligo 5653	GGGAGACAAGAAUAAACGCUCAGCUUCAACUGAUGCUCAGAUCUCUCCAAUGUGG GCAUUCGCUCCGGGUUGACGUUUCGACAGGAGGCUCACAACAGGC
Oligo 5654	GGGAGACAAGAAUAAACGCUCGACCGUUCAGAAAACGAACAGUCUAGUGUCGUCG ACAUUCGCUCCCGGUGAUGCUUUCGACAGGAGGCUCACAACAGGC
Oligo 5662	GGGAGACAAGAAUAAACGCUCGCUCAUUAACAUUGCUUAUGAGCGCGUCAUAAUG ACAUUCGCUCCCAUUAUGGGCUUCGACAGGAGGCUCACAACAGGC
Oligo 5663	GGGAGACAAGAAUAAACGCUCGCUGUGCAUGCAAAUGAUCCAGCAAGUAGCACAG UCAUUCGCUCCCUGUGAUGCUUUCGACAGGAGGCUCACAACAGGC
Oligo 5664	GGGAGACAAGAAUAAACGCUCCACACGCCACUCUCCAAUGGCUGUGACAGCAAUC UCAUUCGCUCCAGAUUGAGGCUUCGACAGGAGGCUCACAACAGGC
Oligo 5665	GGGAGACAAGAAUAAACGCUCCGUGGAUGCAUCUGAGUACUACGUAGCGCGCUUU UCAUUCGCUCCAUUGCGGGCUUUCGACAGGAGGCUCACAACAGGC
Oligo 5666	GGGAGACAAGAAUAAACGCUCUCGAGUCACGAAACAUGCCUCGUCGCUCACGCGU ACAUUCGCUCCACGUAUGAGCUUCGACAGGAGGCUCACAACAGGC
Oligo 5667	GGGAGACAAGAAUAAACGCUCUACGCUAGCCACAACGGCAGCUAUACGAUGCUCG ACAUUCGCUCCCGAGCUGAGUUUCGACAGGAGGCUCACAACAGGC
Oligo 5668	GGGAGACAAGAAUAAACGCUCAGCGCUACAUGAAAAUGUAAUGUUUACUGACAAG ACAUUCGCUCCCUUGUGAGUAUUCGACAGGAGGCUCACAACAGGC
Oligo 5669	GGGAGACAAGAAUAAACGCUCGUUGCGGAACUAAAUAUCGUAACGGAACUACAAG UCAUUCGCUCCCUUGUAGGGCUUCGACAGGAGGCUCACAACAGGC
58PYO1A	GGGCCAAAGAGCACUCCAUUCGAUGGUCGUUUUUCGCACAUUCGCUCCGCGAUAG ACG
57PYO3A	GGGACCGUUCAGAAAACGAACAGUCUAGUGUCGUCGACAUUCGCUCCCGGUGAUG CU
49BRC1A	GAGACGGUCGGGUCCAGAUAUUCGUAUCUGUCGAGUAGAGUGUGGGUC
15THR1A	GGTTGGTGTGGTTGG
20THR2A	GGTTGGTGTGGTTGGCAACC
32THR4A	GGTAGGGCAGGTTGGGTGTTTTCACTTTTGGG
97SPN2A	GGGAUGUAACUGAAUGAAAUGGUGAAGGACGGGUCCAGUAGGCUGCUUCGGCAGC CUACUUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUUACAUC

Table S2. Conditions of selection in the SELEX protocol

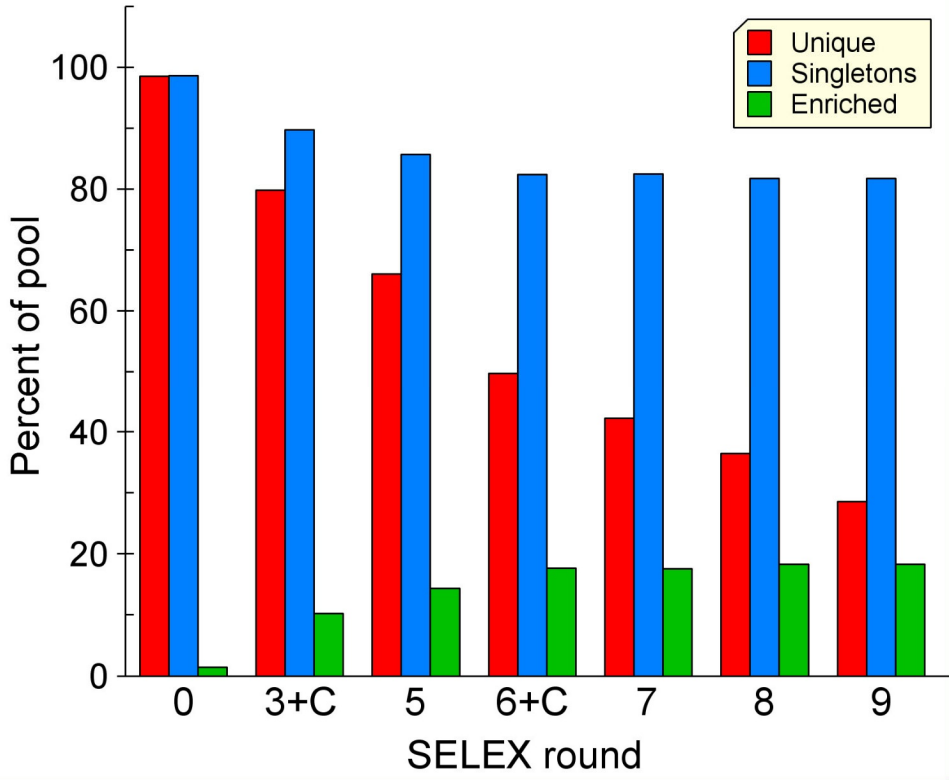
Round	2' FY-RNA: PVD-Pf5	Target	Length of capture oligo (nt)	Incubation temperature (ºC)
1	1:1	16 µM PVD-Pf5	6	4
2	2:1	5.5 µM PVD-Pf5	6	4
3	3:1	3.5 µM PVD-Pf5	7	4
Counter selection	1:1	10.5 µM ENB and 10.5 µM ORB	7	4
4	5:1	3.5 µM PVD-Pf5	8	4
5	6:1	1 µM PVD-Pf5	8	23
6	7:1	0.6 µM PVD-Pf5	8	23
Counter selection	1:1	3.2 µM ENB and 3.2 µM ORB	8	23
7	8:1	0.4 µM PVD-Pf5	8	23
8	10:1	0.2 µM PVD-Pf5	9	23
9	10:1	0.2 µM PVD-Pf5	9	23

Legend: All siderophores (PVD-Pf5, ENB and ORB) were in the ferric form. PVD-Pf5 (pyoverdine Pf5), ENB (enterobactin) and ORB (ornibactin).

4 Supplementary Figures

4.1 Figure S1

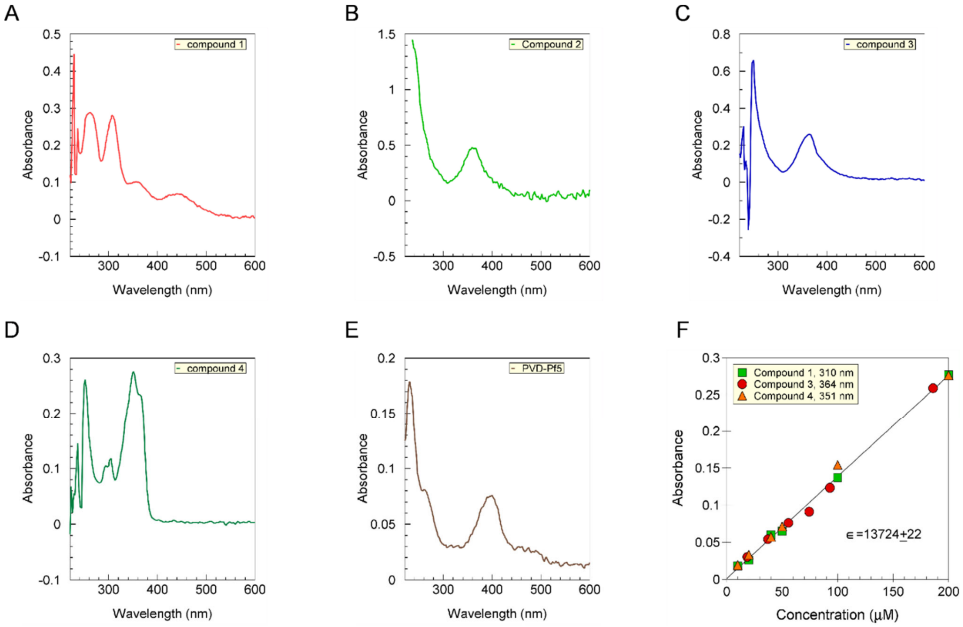
Analysis of NextGen sequencing results



LEGEND:. The percent of each round identified as the unique fraction (number of species in pool divided by pool size), Singletons (species present only once in the database) and Enriched species (species present more than once in the database) is plotted for the original pool (round 0) and rounds 3 through 9. Rounds 3 and 6 include counterselections that were performed after the identified selection round.

4.2 Figure S2

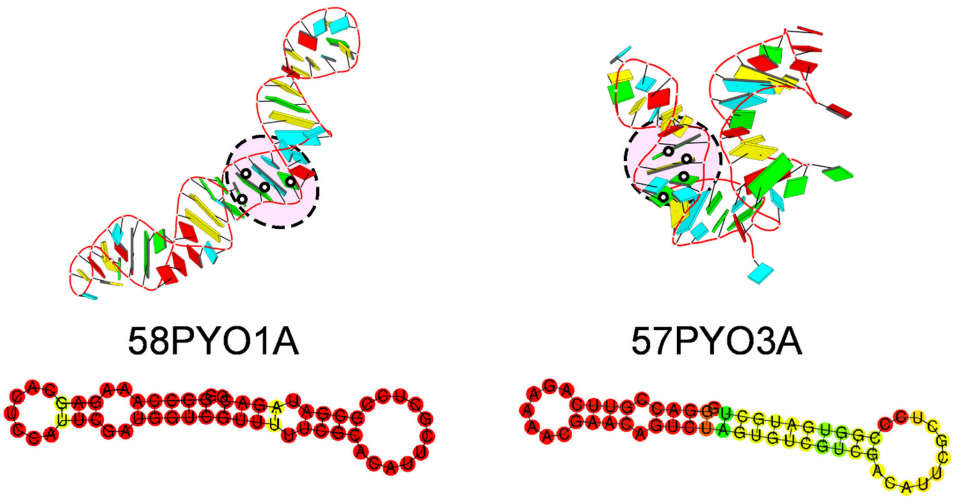
uv/vis absorption spectra of synthesized compounds 1-4



LEGEND: A-E) UV-Vis spectra of compound 1, 2, 3, 4 dissolved in DMSO and PVD-Pf5 dissolved in water, F) Concentration vs absorbance at maximum (310nm, 359nm, 364nm, 351nm, and 400nm for 1, 2, 3, 4 and PVD-Pf5 respectively) to determine the extinction coefficients of compounds 1, 3 and 4.

4.3 Figure S3

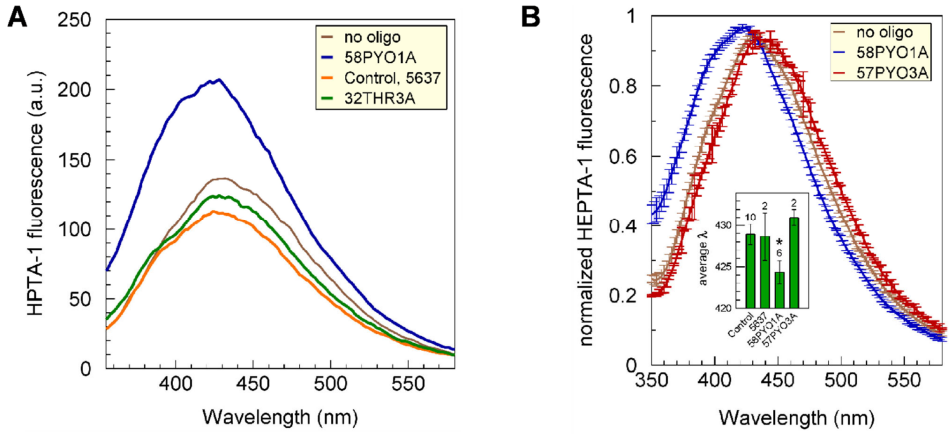
Predicted structures



LEGEND: Top: 3D structures of 58PYO1A and 57PYO3A predicted using 3dRNA and visualized using x3DNA. The black circles identify the positions of Gs proposed to form a G-quartet. **Bottom:** 2D structural renditions predicted by RNA fold.

4.4 Figure S4

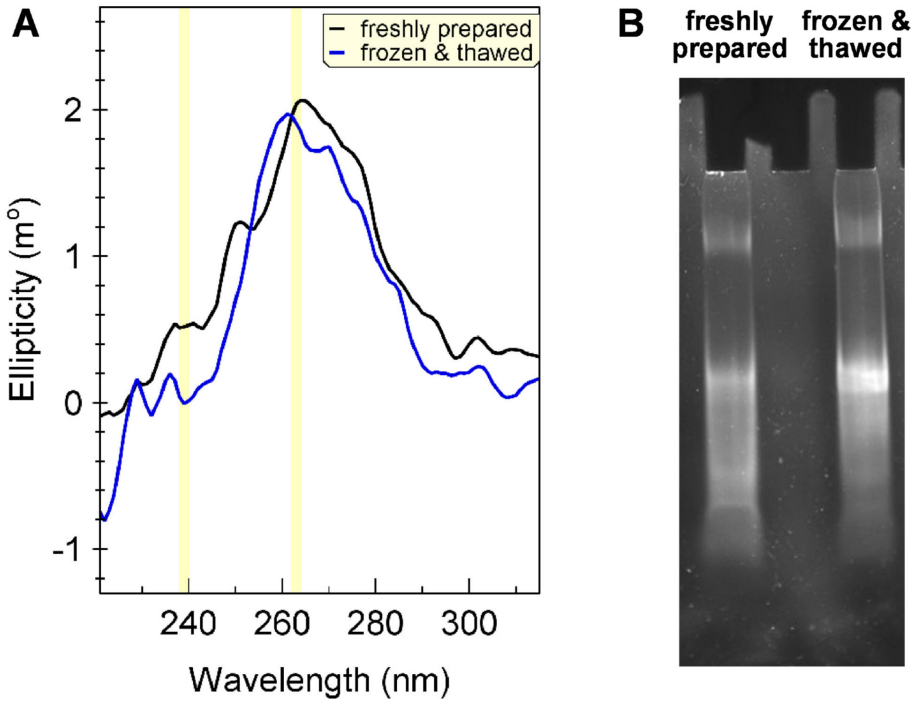
The effect of aptamers on the fluorescence spectrum of HPTA-1



LEGEND: A. The fluorescence of spectra (λ^{ex} = 304 nm and λ^{em} scan (350-600 nm) of 6 μM HPTA-1 alone and in combination with the 2 μM identified oligonucleotides are plotted with from 340-590 nm. Single smoothed spectra are shown. **B.** The fluorescence of spectra (λ^{ex} = 304 nm and λ^{em} scan (350-600 nm) of 6 μM HPTA-1 alone and in combination with the 2 μM 58PYO1A or 57PYO3A. Individual spectra were first normalized to the maximum peak height for each spectrum then independently obtained normalized spectra were averaged for each condition and the averaged spectra smoothed. Contributing numbers of independent spectra were "no oligonucleotide" (10), 58PYO1A (6) and 57PYO3A (2). The SEM for the spectral averages are shown.

4.5 Figure S5

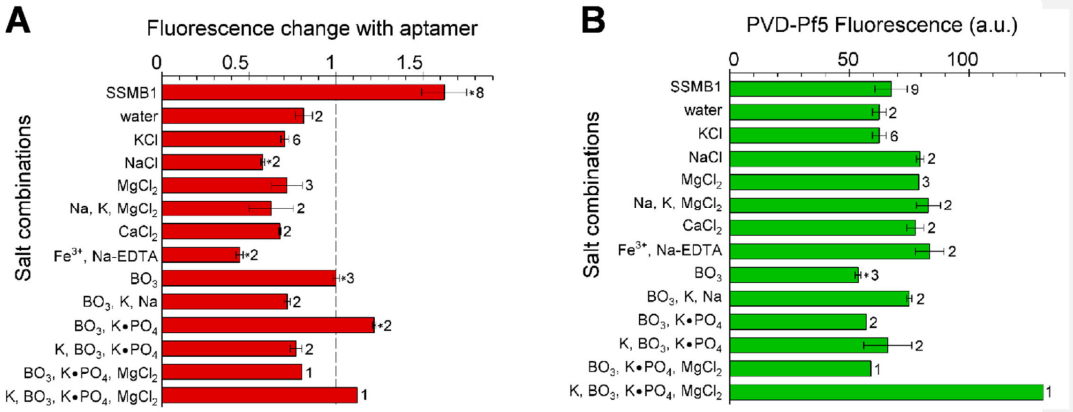
The effect of freeze-thaw on the CD spectrum and molecular size of 57PYO3A



LEGEND:. **A.** CD spectra 2uM of 57PYO3A for freshly prepared and frozen thawed (-20 ºC for 18 h then to 23 ºC) sample in SSMB1. Each spectrum is the smoothed average of two independent CD spectral collections of 3 scans each. B. nondenaturing gel electrophoresis of 57PYO3A when freshly prepared and after freeze-thaw.

4.6 Figure S6

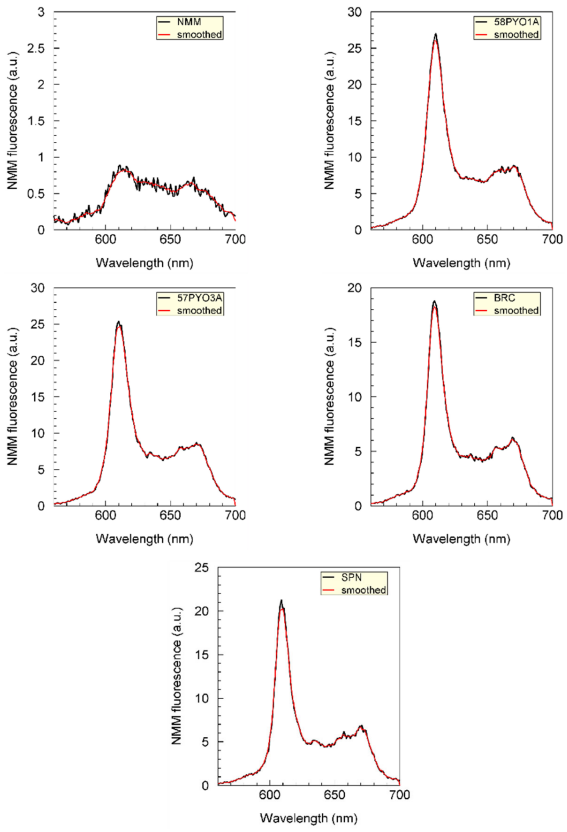
The effect of salt combinations on PVD-Pf5 binding to 57PYO3A



LEGEND: A. For each salt combination, the maximum PVD-Pf5 fluorescence (measured at 460–466 nm, depending on the spectrum) in the presence of 400 nM aptamer was divided by the maximum fluorescence in its absence. The mean ratio from all experiments for each salt mix is shown as red bars, with error bars indicating the standard error of the mean. **B.** The fluorescence of PVD-Pf5 in the absence of aptamer is shown for each salt mixture in A. Statistical significance relative to water alone was assessed using the Student's *t*-test ($p < 0.05$). Numbers to the right of the bars indicate the number of independent measurements used to calculate each mean. Phosphate was supplied as KH_2PO_4, abbreviated here as $K{\cdot}PO_4$.

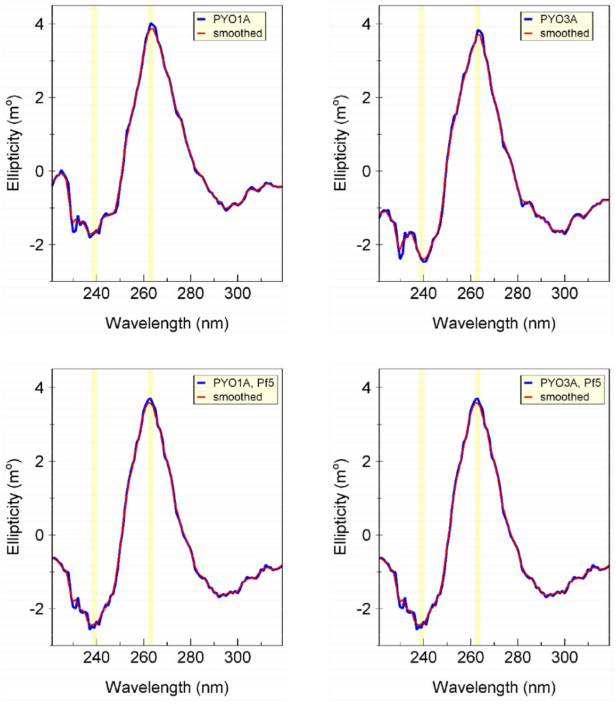
4.7 Figure S7

Raw data for NMM spectra in Fig. 4D with and without smoothing



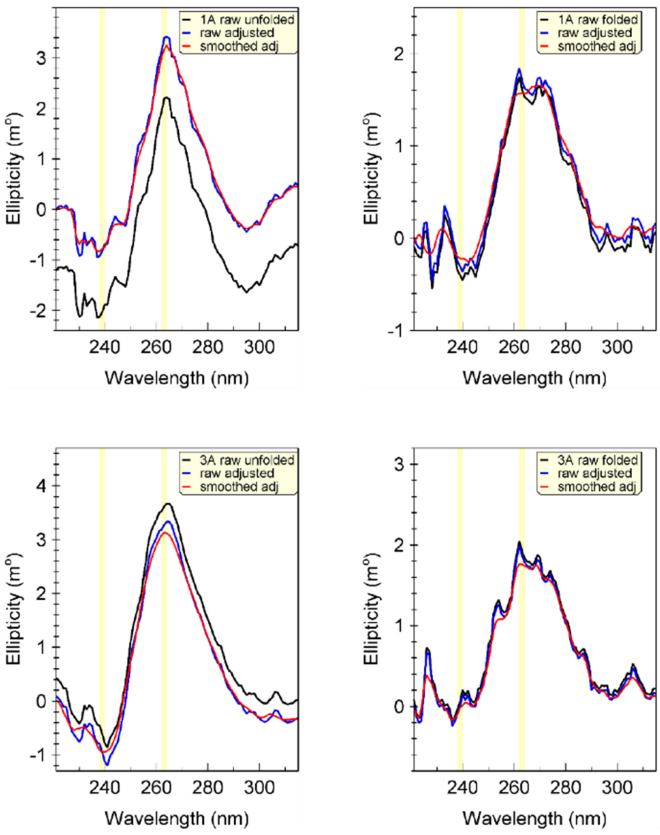
4.8 Figure S8

Raw data for CD spectra in Fig. 4E with and without smoothing



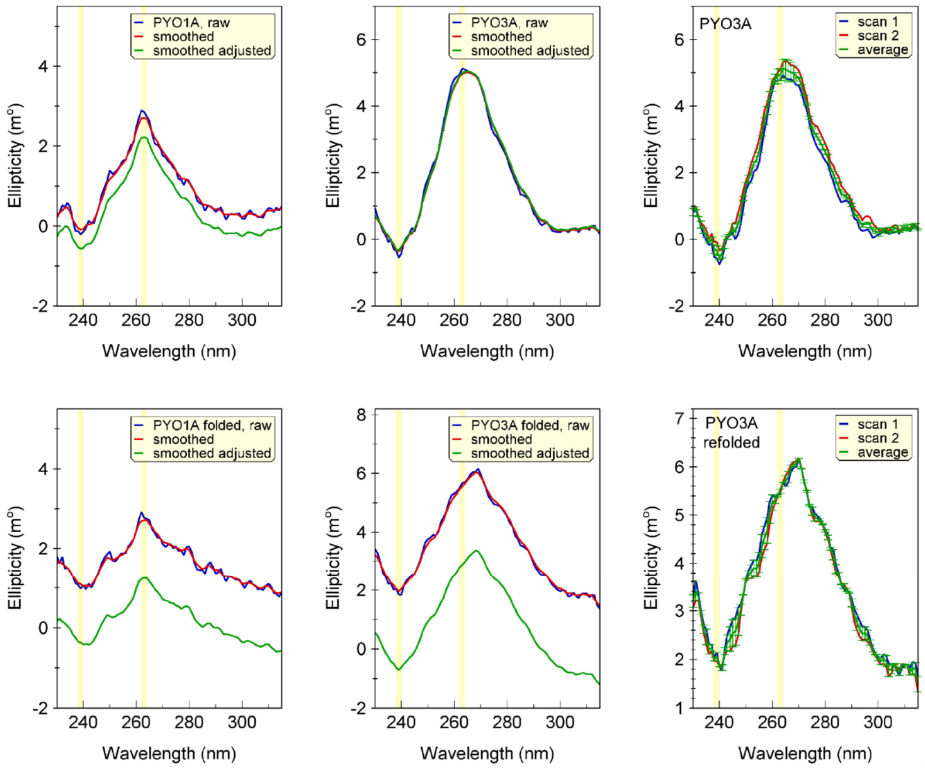
4.9 Figure S9

Raw data for CD spectra in Fig. 5B with and without baseline adjustments and smoothing



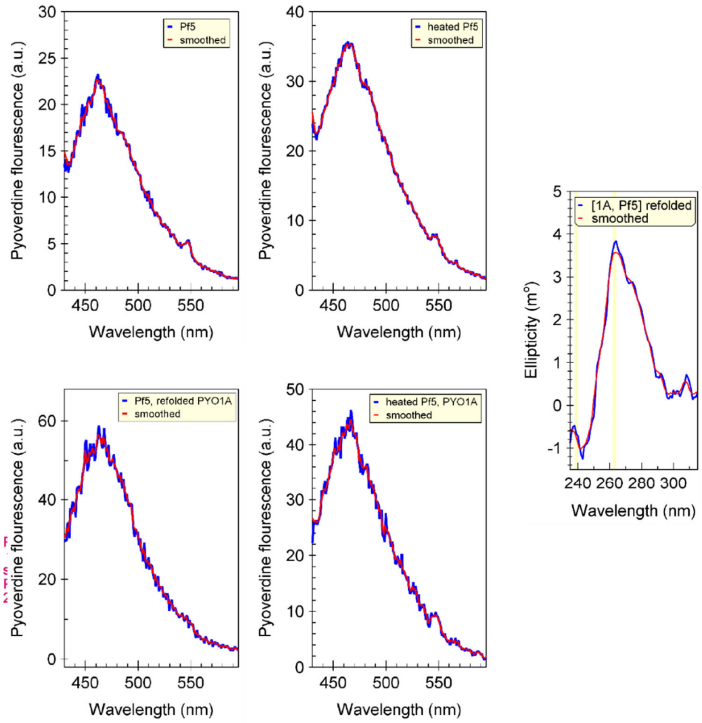
4.10 Figure S10

Raw data for CD spectra in Fig. 5C with and without baseline adjustments and smoothing



4.11 Figure S11

Raw data for CD spectra in Fig. 5C with and without baseline adjustments and smoothing



4.12 Figure S12

Raw data for CD spectra in Fig. 6C with and without smoothing

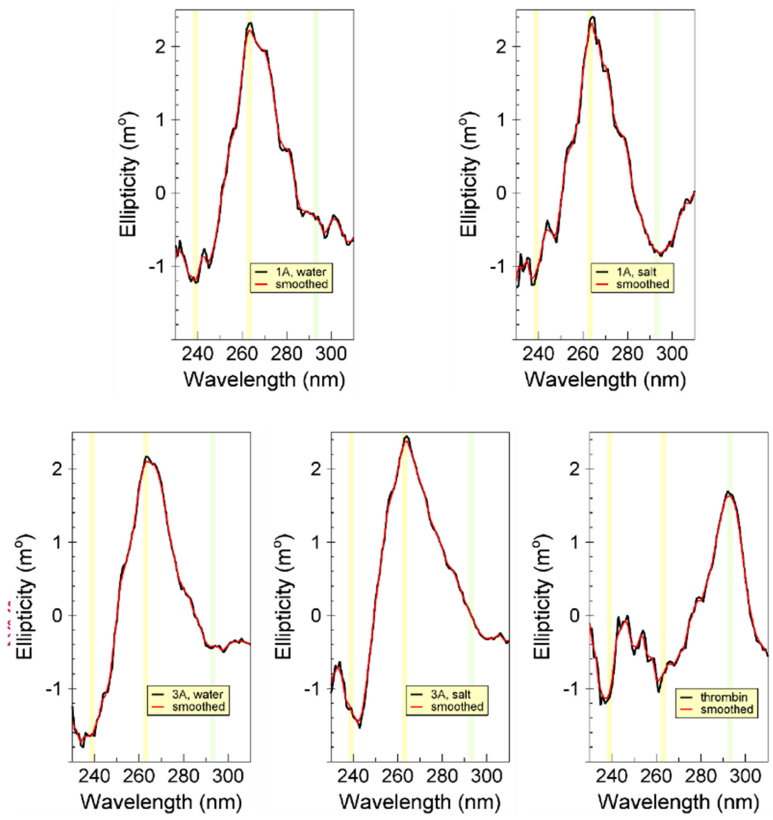
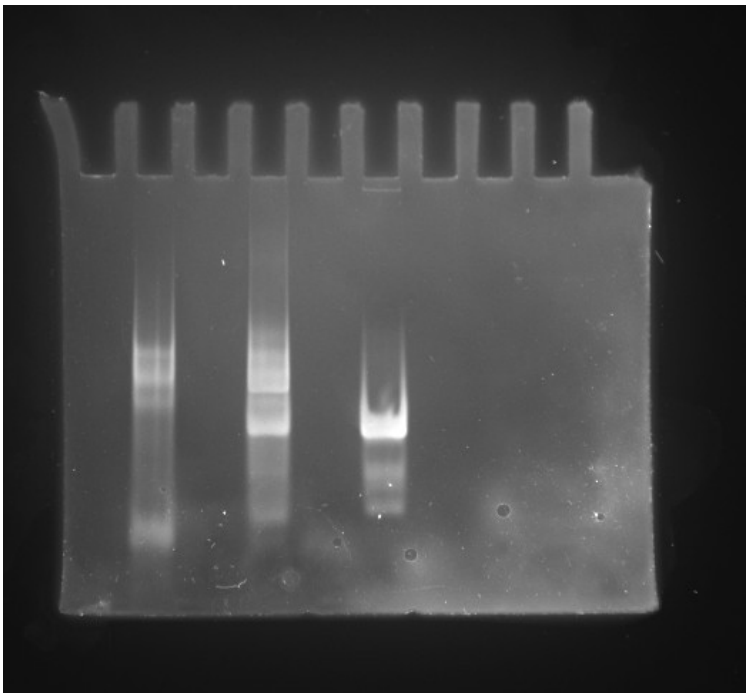


Figure S13

Full gel image for Figure 4G



5 Citations

1. Levine, H.A., and Nilsen-Hamilton, M. A mathematical analysis of SELEX. *Comput Biol Chem* **31,** 11-35 10.1016/j.compbiolchem.2006.10.002 (2007).
2. Hoinka, J., Backofen, R., and Przytycka, T.M. AptaSUITE: A Full-Featured Bioinformatics Framework for the Comprehensive Analysis of Aptamers from HT-SELEX Experiments. *Molecular therapy. Nucleic acids* **11,** 515-517 10.1016/j.omtn.2018.04.006 (2018).