Non-covalent Dyes in Microscale Thermophoresis for Studying RNA Ligand Interactions and Modifications

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RNA and DNA oligo sequences

Table S1. Oligos used for RNA-MST experiments. **Category 1**: no structure, <10mer; **category 2**: no structure, 10–15mer; **category 3**: no structure, 16–20mer; **category 4**: hairpin, 16–20mer; **category 5**: bulge/internal loop + hairpin, 21–25mer; **category 6**: internal loop + hairpin, 26–30mer; **category 7**: bulge + internal loop + hairpin(s), 31–50mer; **category 8**: 2 internal loops + 1 hairpin, 50mer; **category 9**: 3 internal loops + 1 hairpin, 60mer; **category 10**: various functional RNA.

OligoID	Cat.	RNA sequence	Manufacturer
MH993	1	GUUAUUC	Iba
MH996	1	UUUUAUUUU	Biomers
MH474	1	GGUACCCAA	Iba
MH122	2	ACAGCUAUCCAUUG	Iba
MH847	2	UCUGAGGGUCCAGGG	Biomers
MH268	2	CCCGGUAAUCGCAUA	Salifu
MH266	3	AAACUUAAAACUUUACUGU	Salifu
MH907	3	ACAAGAAGGGUUUGAUCAUC	Iba
MH272	3	AGAAAUAUGUCUGAUAAA	Salifu
MH355	4	CGCGGGAGACCGGGGUUCGA	Dharmacon
MH261	4	GCUUGAAACCAGCUUUGGG	Salifu
MH1428	4	GGAUCCUCUAGAGUCGACCU	Idt
MH515	5	GGUGGGGUUCCCGAGCGGCCAAAG	Iba
MH499	5	GUUCGAUCCACAGAAUUCGCACCA	Biomers
MH649	5	GGUUCGAUUCCCCGACGGGGAGCCA	Iba
MH857	6	UCUAGAGGAUCCCGGGUACCGAGCUC	Biomers
MH876	6	UGCCGCCCGUCUUGAAACACGGACCAAGGA	Iba
MH1145	6	AACUUAAUGUUUUUGCAUUGGACUUUGAGUUA	Iba
MH761	7	GGCGUGUAGGAUAUUAAAUCAUACCAGAUCGCGG	Iba
MH646	7	UCCUCGUUAGUAUAGUGGUGAGUAUCCCCGCCUAUAUC	Iba
MH566	7	GUCACGCGGGAGACCGGGGUUCGAUUCCCCGACGGGGAGCCA	Iba
MH723	8	CCGCGAUCUGGAGAGGUGAAGAAUACGACCACCUACAAGAAGGACACGCC	Iba
MH721	8	CCGCGAUCUGGAGAGGUGAAGAAUACGACCACCUAAGAAGGACACGCC	Iba
MH762	8	CCGCGAUCUGGAGAGGUGAAGAAUUCGACCACCUACCAGAAGGACACGCC	Iba
MH950	9	CGGAGCCAGCGAGUCUAACCUUGGCCGAGAGGUCUUGGUAAUCUUGUGAAACUC CGUCGU	Iba

| Name	#	Sequence	Source
MH952	9	GGCCUCAGGAUCUGCUUAGAGAAGGGGGCAACUCCAUCUCAGAGCGGAGAAUUUGGACAA	Iba
MH953	9	UCUUGGAUUUAUUGAAGACUAACUACUGCGAAAGCAUUUGCCAAGGACGUUUUCAUUAAU	Iba
tRNA^{Asp}	10	UCCUCGUUAGUAUAGUGGUGAGUAUCCCCGCCUGUCACGCGGGAGACCGGGGUUCGAUUCCCCGACGGGGAG	In house IVT
PreQ$_1$ RS (*Thermoanaerobacter tengcongensis*)	10	GGAGAGGUUCUAGCUACACCUCUAUAAAAAACUAA	biomers
SAM-I RS (*Thermoanaerobacter tengcongensis*)	10	GGCUUAUCAAGAGAGGGGGAGCGACUGGCGCGAAGAACCCCGGCAACCAGAAAUGGUGCCAAUUCCUGCAGCGGAAACGUUGAAAGAUGAGCCG	In house IVT
SAM-VI RS (*Bifidobacterium angulatum*)	–	GGCAUUGUGCCUCGCAUUGCACUCCGCGGGGCGAUAAGUCCUGAAAAGGGAUGUC	In house IVT
Cy5-PreQ$_1$ RS (*Bacillus subtilis*)	–	Cy5-AGAGGUUCUAGCUACACCCUCUAUAAAAAACUAA	Eurofins Genomics
Cy5-SAM-VI RS (*Bifidobacterium angulatum*)	–	Cy5-GGCAUUGUGCCUCGCAUUGCACUCCGCGGGGCGAUAAGUCCUGAAAAGGGAUGUC	Eurofins Genomics
AdoCbl riboswitch (Marine metagenome *env8* 4WJvar)	–	GGCCUGAAAGCGUGGUGGGGAAGUGACGUGAAAUUCGUCCAGAUUACUUGAUACGGUUAUACUCCGAGUGCCACCCAGGCCAUACAACGAGCAAGGAGACUC	GenScript
Fluoride riboswitch (*Thermotoga petrophila*)	–	GGGCGAUGAGGCCCGCCCAAACUGCCCUGAAAAGGGCUGAUGGCCUCUACUG	GenScript
Neomycin aptamer (synthetic)	–	GGCUGCUUGUCCUUUAAUGGUCCAGUC	GenScript
METTL3/14 substrate	–	AACUUAAUGUUGCAUUGGACUUGAGUUA	Iba

Table S2. DNA templates and primers used for PCR and IVT.

| OligoID	description	DNA sequence	Manufacturer
MH53	Universal forward primer	CGCGCGAAGCTTAATACGACTCACTATA	IDT
EK0004	Reverse primer SAM-I RS	CGGCTCATCTTTCAACGTTTCCG	IDT
EK0010	Reverse primer SAM-VI RS	GACATCCCTTTTCAGGACTTATCGCCCCG	IDT
MH1102	Reverse primer tRNA^{Asp}	TGGCGGGCCGTCG	Iba
EK0003	Template SAM-I RS	CGGCTCATCTTTCAACGTTTCCGCTGCAGGAATTGGCACCATTTCTGGTTGCCGGGGTTCTTCGCGCCAGTCGCTCCCCCTCTCTTGATAAGCCTATAGTGAGTCGTATTA	IDT
EK0009	Template SAM-VI RS	GACATCCCTTTTCAGGACTTATCGCCCCGCGGAGTGCAATGCGAGGCACAATGCCTATAGTGAGTCGTATTA	IDT
MH1101	Template tRNA^{Asp}	TGGCGGGCCGTCGGGGAATCGAACCCCGGTCTCCCGCGTGACAGGCGGGGATACTCACCACTATACTAACGACCCTATAGTGAGTCGTATT	Iba

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Supplementary Figures
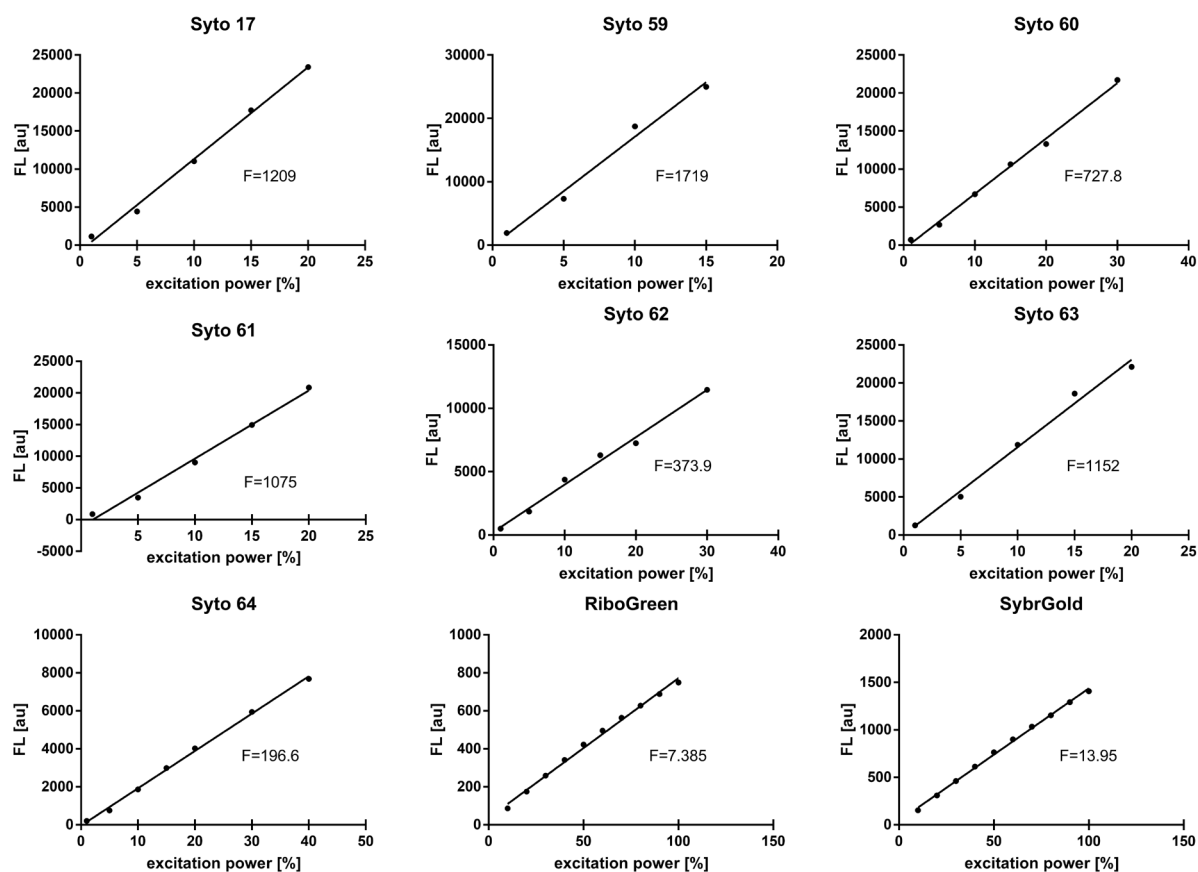
Figure S1. Regression plots for dye fluorescence normalization. Each dye (1×) was mixed with 100 nM of MH646 as RNA model oligonucleotide (UCCUCGUUAGUAUAGUGGUGAGUAUCCCCGCCUAUAUC). The initial fluorescence intensity as a function of the excitation power was determined pre-MST with either the NanoBlue laser for SybrGold and RiboGreen or the PicoRed laser for the Syto dyes. Linear regression yielded a slope-derived sensitivity factor (F) for the normalization of fluorescence intensity during structure-activity-relationship experiments.
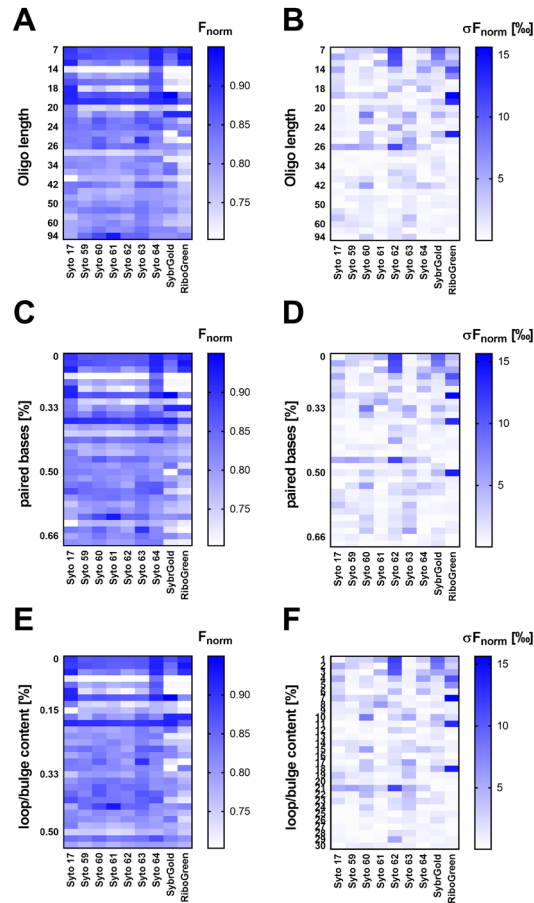
Figure S2. Structural RNA-dependent effects on F_{norm}. (**A**) Correlation between oligo length and F_{norm}. (**B**) Correlation between oligo length and σF_{norm}. (**C**) Correlation between hybridized base content and F_{norm}. (**D**) Correlation between hybridized base content and σF_{norm}. (**E**) Correlation between loop/bulge content and F_{norm}. (**F**) Correlation between loop/bulge content and σF_{norm}.
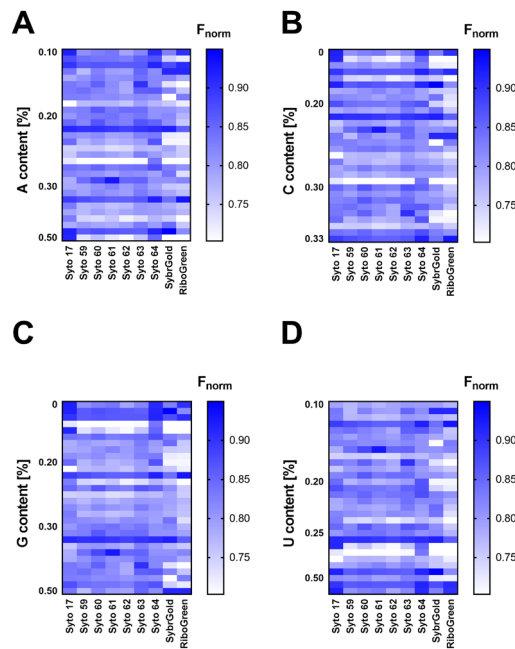

Figure S3. RNA sequence-dependent effects on F_{norm}. (**A**) Correlation between adenine content and F_{norm}. (**B**) Correlation between cytosine content and F_{norm}. (**C**) Correlation between guanine content and F_{norm}. (**D**) Correlation between uridine content and F_{norm}.
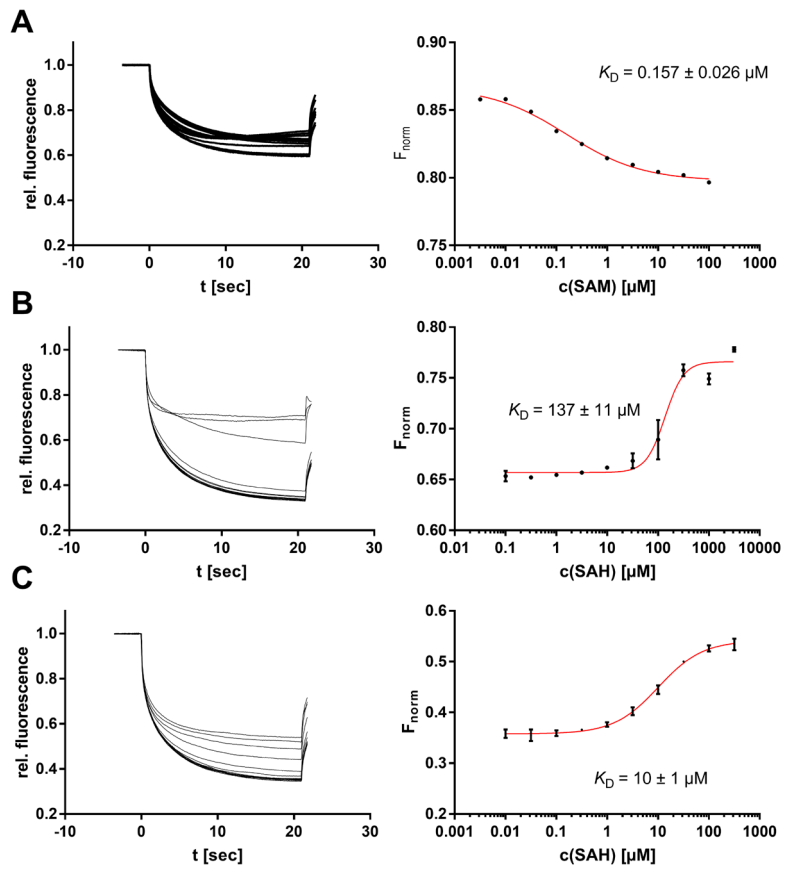
Figure S4. Analysis of RNA-ligand interaction by in situ labelling with SybrGold (1×). (**A**) Interaction between SAM-I RS and SAM (F_{norm} at 1 s). (**B**) SAM-I RS and SAH (F_{norm} at 1 s). (**C**) SAM-VI RS and SAH (F_{norm} at 15 s).
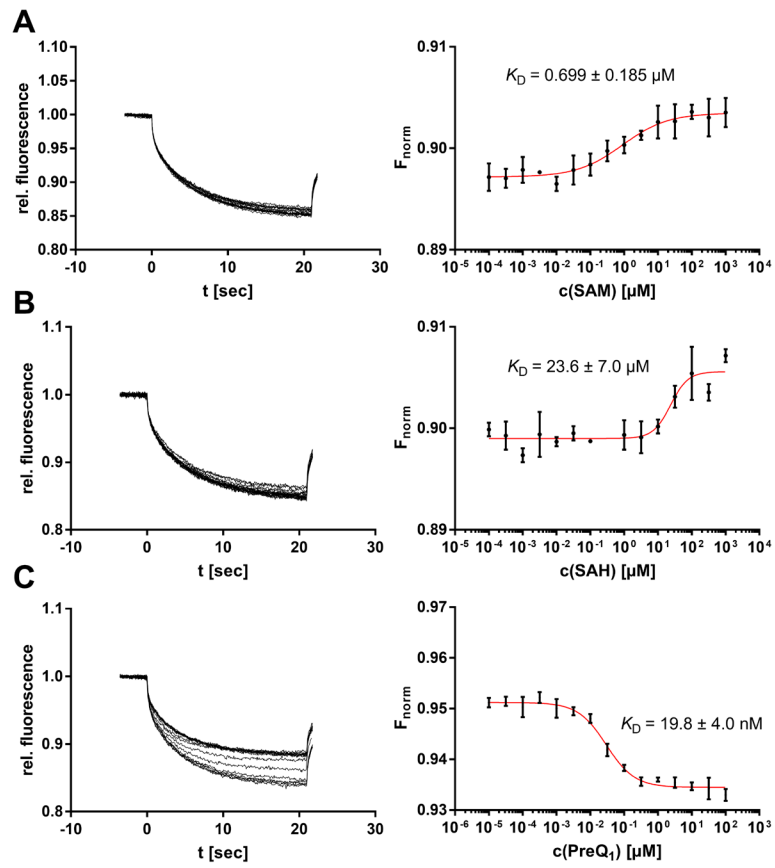
Figure S5. Analysis of RNA-ligand interaction by Cy5-labelled RNA-MST (**A**) Interaction between SAM-VI RS and SAM (F_{norm} at 5 s). (**B**) SAM-VI RS and SAH (F_{norm} at 5 s). (**C**) PreQ$_1$ RS and PreQ$_1$ (F_{norm} at 1 s).
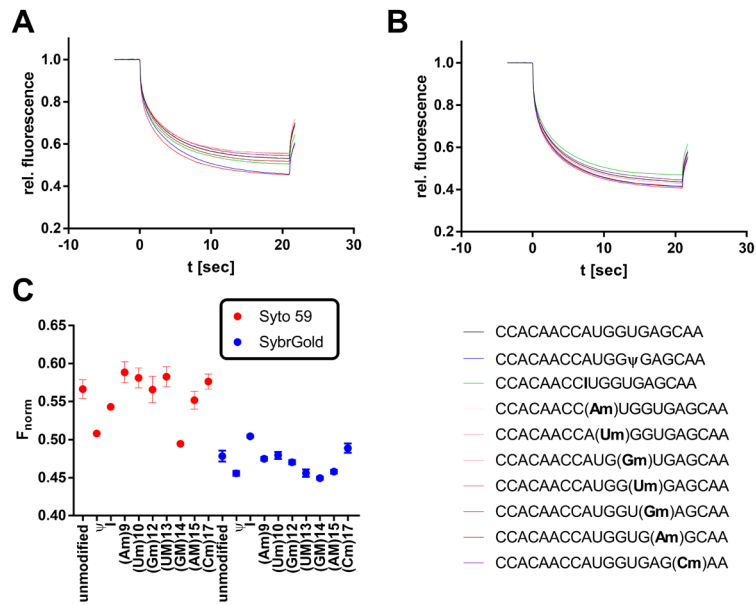
Figure S6. Analysis of various modifications of the model oligo CCACAACCAUGGUGAGCAA (100 nM) by MST. (**A**) Thermophoresis curves for the usage of Syto 59 as a reporter dye. (**B**) Thermophoresis curves for the usage of SybrGold as a reporter dye. (**C**) MST response (F_{norm} at 10 s) of the modified oligos in comparison to their unmodified analogues.
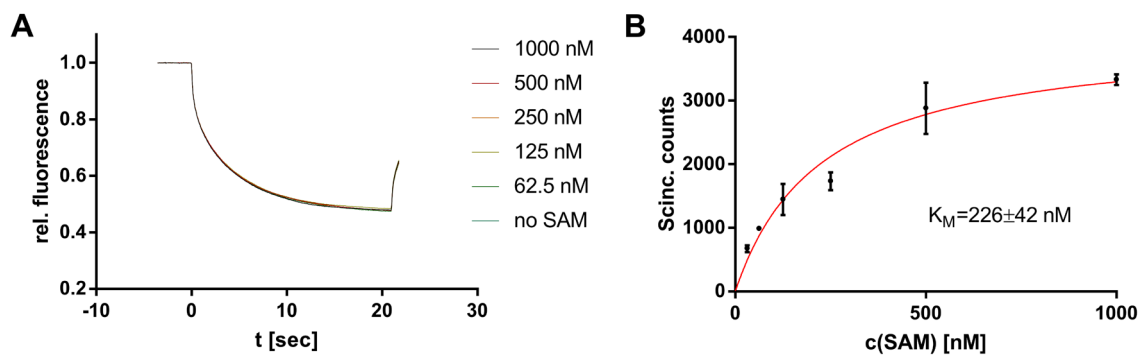
Figure S7. Supplementary data for the establishment of the METTL3/14 assay. (**A**) Thermophoresis curves for the METTL3/14 reaction inhibited by the initial addition of SAH (10 µM). Enzymatic substrate methylation with varying concentrations of SAM as the cofactor. After 2 h of incubation time, the crude reaction mixture was supplemented with the anti-m6A antibody and analyzed by SybrGold MST. (**B**) Tritium-based METTL3/14 methyltransferase assay. The Michaelis-Menten plot was derived from radiometric analysis of 3H incorporation into RNA substrate AACUUAAUGUUUGCAUUGGACUUGAGUUA. A K_M value (226 nM) was calculated by non-linear regression (Michaelis Menten).
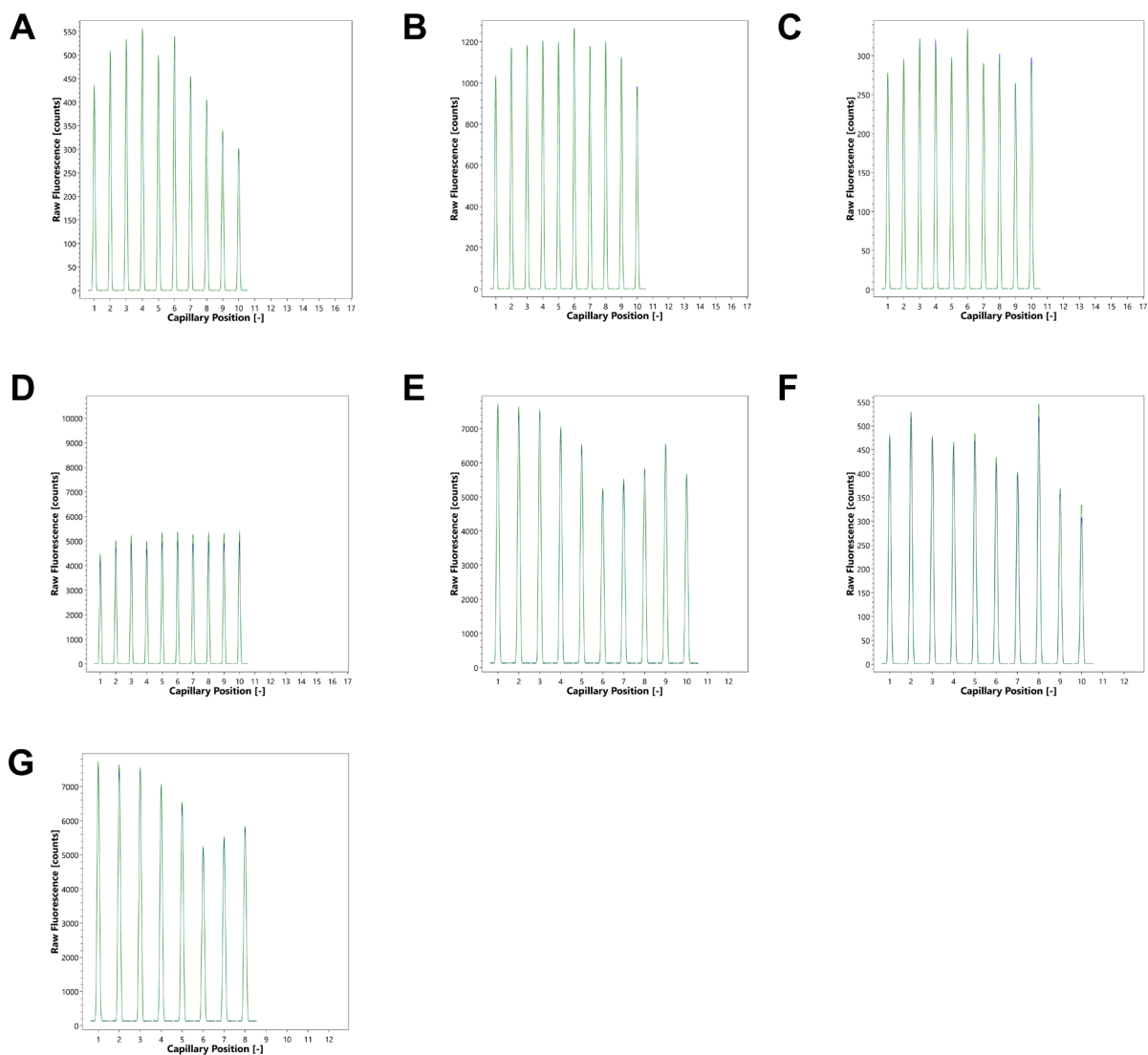
Figure S8. Capillary scans of RNA-ligand interaction samples for the assessment of dye displacement by ligand binding. Capillary scans are presented with increasing concentration corresponding to the MST curves in Figure 4 and Figure S4. (**A**) PreQ$_1$ RS vs. PreQ$_1$. (**B**) SAM-I RS vs. SAM. (**C**) SAM-VI RS vs. SAM. (**D**) Fluoride RS vs. potassium fluoride. (**E**) AdoCbl RS vs. adenosyl cobalamin. (**F**) DNMT2 vs. tRNA. (**G**) Neomycin aptamer vs. neomycin.