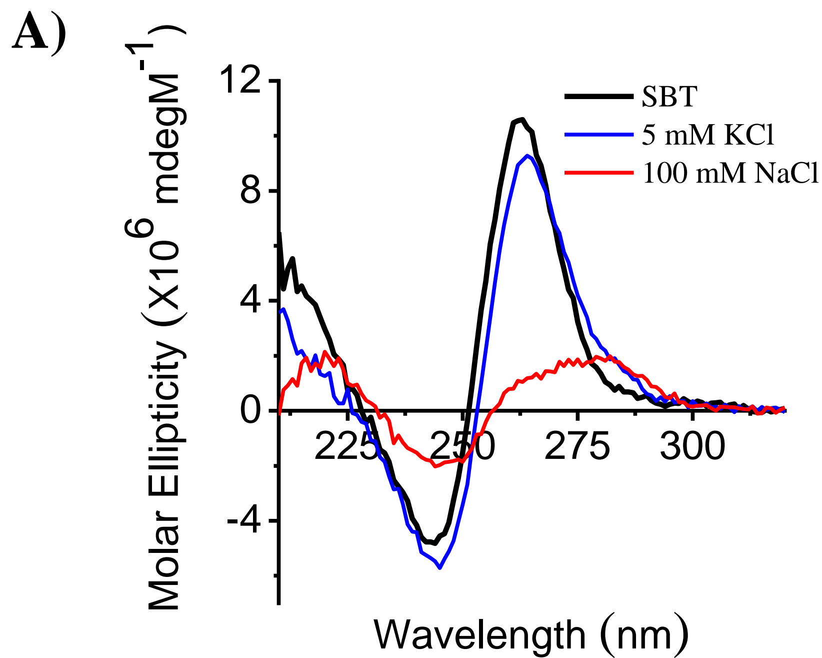
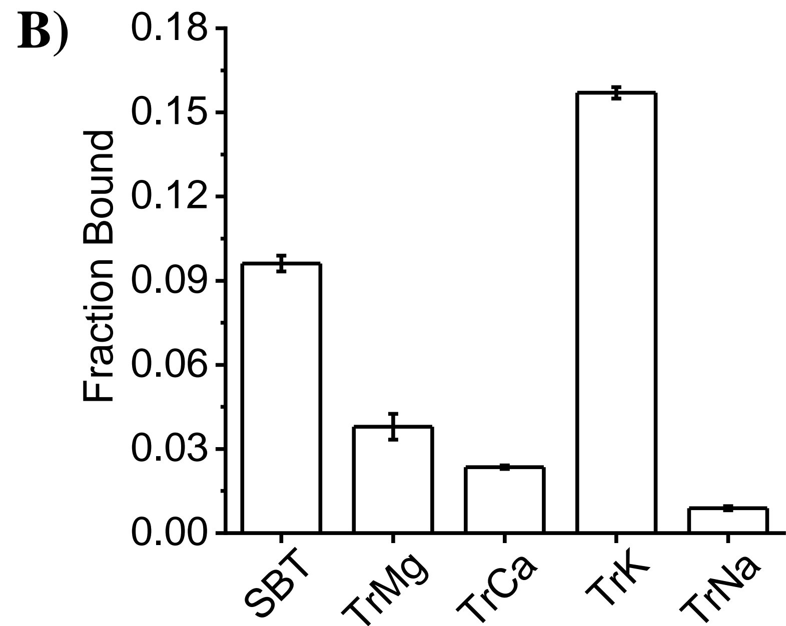
SI Table 1: Summary of the sequences obtained from several SELEX experiments done to isolate an ssDNA aptamer for cAMP. Sequences under PreNSE were eluted prior to nonspecific elimination, whereas sequences under PostNSE were eluted after nonspecific elimination. Libraries A-C are DNA libraries obtained from three independent synthesis. Sequences are divided into groups A-E based on multiple sequence alignment of randomized regions. Note that sequences in Group E were predominantly obtained PreNSE, and hence are nonspecific and excluded from further study.

	Library	Solid Support	Fraction Bound	Group A	Group B	Group C	Group D	Group E	Total
SELEX-1 PostNSE	A	C2'-Biotin-Streptavidin round (1-13) C2'-Agarsose round (14-23)	NA	11	6	10	0	0	27
SELEX-2 PostNSE	B	C2' and C8 agarose	~ 3%	3	22	0	3	0	28
SELEX-3 PreNSE		C2' and C8 agarose	~ 35 %	*1*	*4*	*0*	*0*	*9*	*14*
SELEX-3 PostNSE			~1.5 %	0	12	0	0	1	13
SELEX-4 PreNSE	C	C2, C2' and C8 agarose	~ 50 %	*0*	*1*	*0*	*0*	*16*	*17*
SELEX-4 PostNSE			~ 20 %	3	9	0	0	2	14
Total PostNSE				17	49	10	3	3	82

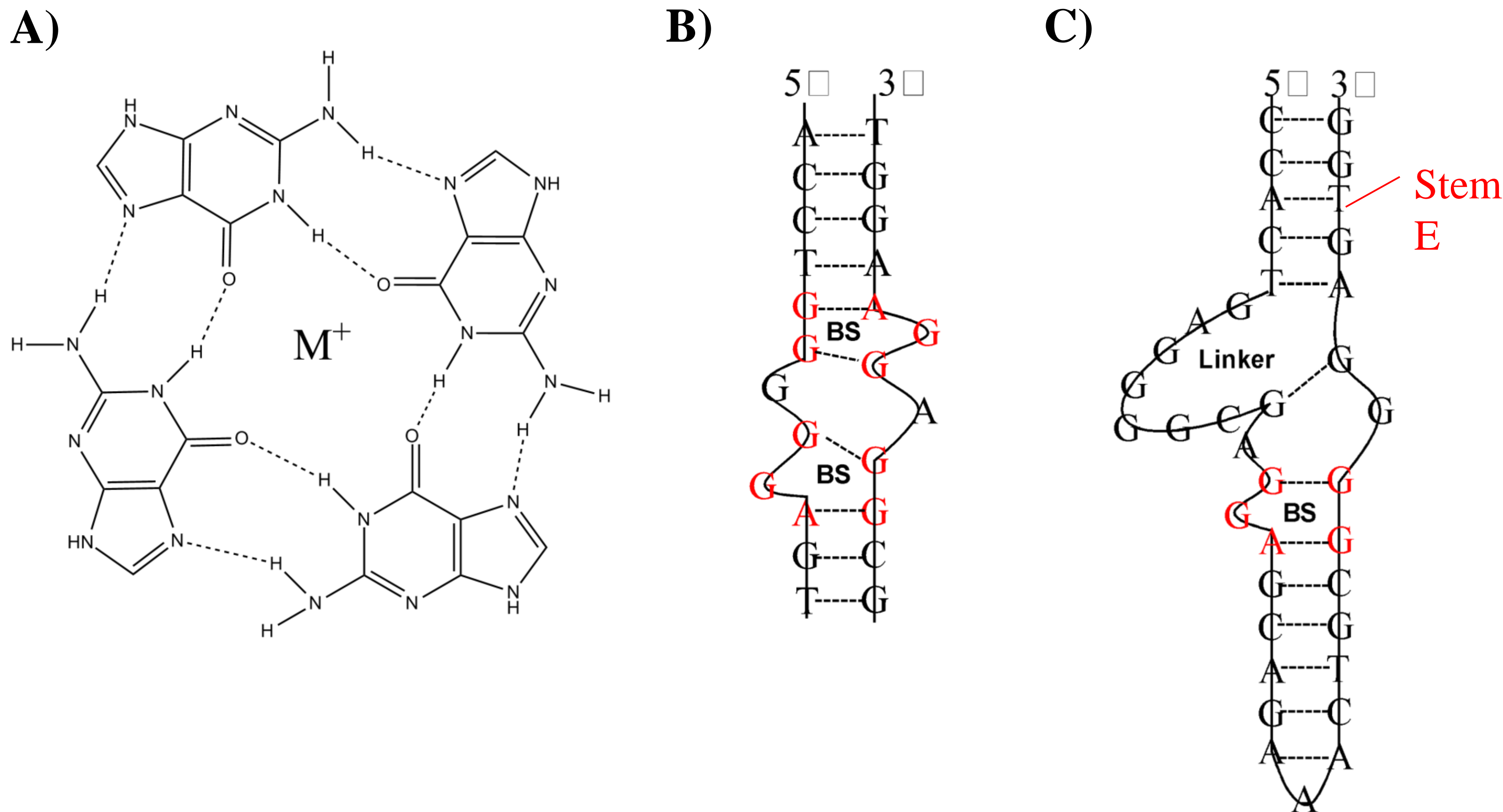
SI Table 2: Nucleic acid sequences used in the study

Name	Sequence (5'-3')	Modification
Full Length	ATACCAGCTTATTCAATTCGAGGCGGGTGGGTGGGTTGAAT ATGCGGATCACGCCACAGATAGTAAGTGCAATCT	None
Apt-A	ATGCGGATCACGCCACAGATAGTAAGTGCAATCT	None
Apt-B	ATACCAGCTTATTCAATTCGAGGCGGGTGGGTGGGTTGAAT	None
Apt-C / caDNAapt-1	ATTCAATTCGAGGCGGGTGGGTGGGTTGAAT	None
Apt-D	TTCGAGGCGGGTGGGTGGGTTGAAT	None
Apt-E	ATTCAATTCGAGGCGGGTGGGTGGG	None
caDNAptT-1	ATTCAATTCGAGGCGGGTGGGTGGGTTGAAT	TMR at 3' terminus
caDNAptT-2	ATTCAATTCGAGGCGGGTGGGTGGGT**T**GAAT	TMR at red T
caDNAptT-3	ATTCAATTCGAGGCGGGTGGGTGGGTTGAAT	TMR at green T
FPL	ATACCAGCTTATTCAATT	None
RPL	AGATTGCACTTACTATCT	None
Library	ATACCAGCTTATTCAATT -N40- GATAGTAAGTGCAATCT	N=A:C:G:T (1.5:1.5:1.0:1.2)

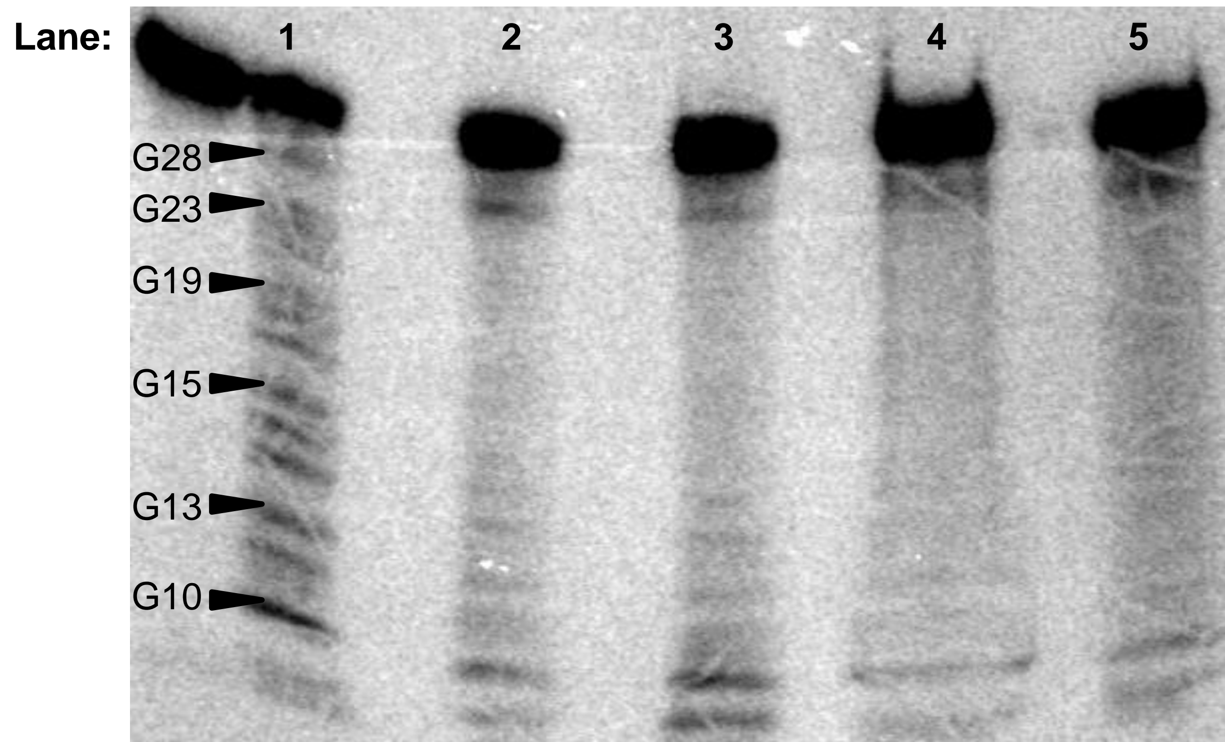




SI Figure 1: **a**) CD spectrum of caDNApt-1 in SBT (black) and in solution containing Tris Cl (20 mM, pH 7.5) and 0.2 % Tween 20 and either 5 mM KCl (blue) or 100 mM NaCl (red) **b**) Fraction bound of 5´-^{32}P labelled caDNAapt-1 on cAMP-agarose beads in SBT and in solutions containing Tris.Cl (20 mM, pH 7.5) and 0.2 % Tween-20 and one of the following: 1mM $MgCl_2$ (TrMg), 1 mM $CaCl_2$ (TrCa), 5 mM KCl (TrK) and 100 mM NaCl (TrNa).

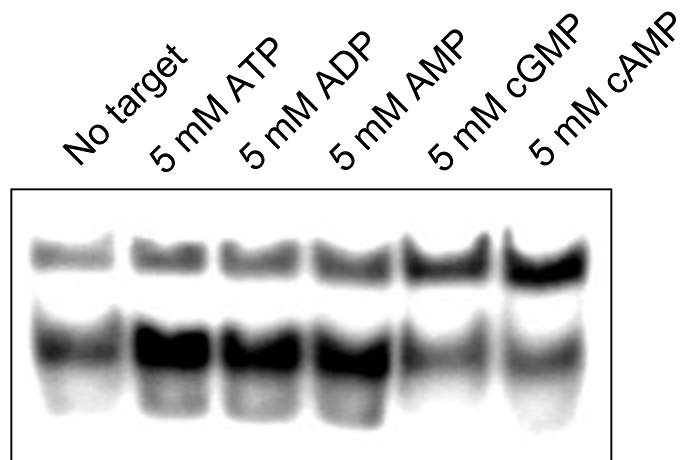


SI Figure 2: a) G-quadruplex base pairing stabilized by metal ions. **b**) Structure of ATP-binding aptamer as revealed by NMR, BS: binding site. **c)** Predicted structure of cAMP-specific adenosine-recognition DNA aptamer isolated by Barbu et al.

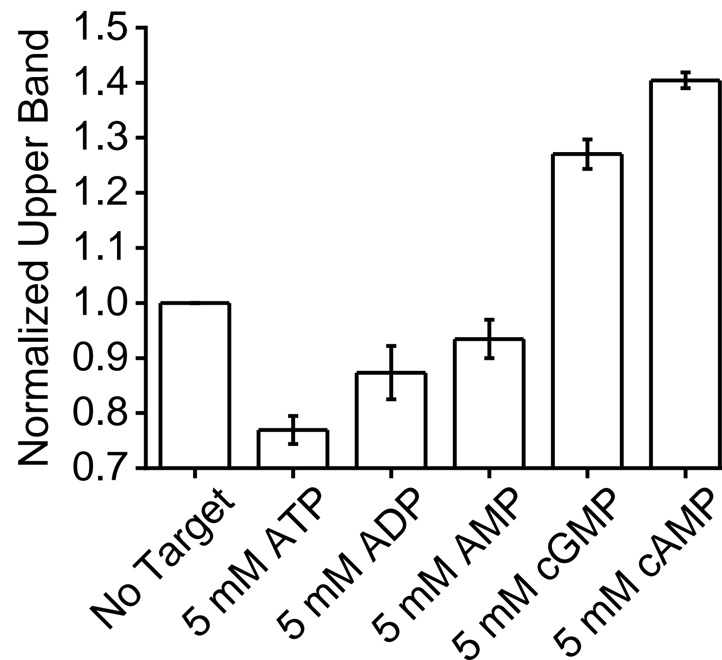


SI Figure 3: DMS-piperidine-cleavage-assay of caDNApt-1 in the presence of excess $MgCl_2$. Products were electrophoresed after performing DMS-mediated methylation and cleavage of 5'-^{32}P -caDNApt-1 in Tris.Cl (1 mM, pH 7.5, lane 1) or SBT with no additives (lane 2), with cAMP (10 mM, lane 3), with $MgCl_2$ (9 mM, lane 4), or with both additives followed. Note that G bases show protection from cleavage in the presence of cAMP and that the $MgCl_2$ necessary for conformation change (Figure 3a) does not interfere in the formation of G-quartet structure.

A)



B)



SI Figure 4: **a**) 12**%** Non denaturing gel electrophoresis using radiolabeled caDNApt-1 (1nM) and unlabeled caDNApt-1 (5 nM) incubated at 25 ºC for 3 h in SBT containing 9 mM $MgCl_2$ in the presence of 5mM ATP/ADP/AMP/cGMP/cAMP as indicated. **b)** Quantification of gel shown in a. Bar graph showing the fraction of the slower band intensity, normalized to the fraction in absence of any of these molecules. Error bars: mean ± s.e.m., n=3.