Supplemental Data

Selection of 2'-Deoxy-2'-Fluoroarabino Nucleic Acid (FANA) Aptamers that Bind HIV-1 Integrase with Picomolar Affinity

By

Kevin M. Rose¹, Irani Alves Ferreira-Bravo¹, Min Li³, Robert Craigie³, Mark A. Ditzler⁴, Phillip Holliger⁵, and Jeffrey J. DeStefano^{1,2*}

¹Cell Biology and Molecular Genetics, Bioscience Research Building, University of Maryland, College Park, MD 20742 USA

²Maryland Pathogen Research Institute (MPRI), College Park, MD 20742 USA

³Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892

⁴Exobiology Branch, Space Science and Astrobiology Division, NASA Ames Research Center,

Moffett Field, CA 94035

⁵MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge CB2 0QH UK

*<u>To whom correspondence should be addressed:</u> Phone: +1 301-405-5449 E-mail: jdestefa@umd.edu



Figure S1. Example of apparent equilibrium dissociation constant $(K_{D,app})$ determination. Standard reactions for $K_{D,app}$ determinations were performed in 1 ml of Binding Buffer (20 mM Tris-HCl pH 7.5, 200 mM NaCl, 6 mM MgCl₂, and 1 mM DTT) with 0.1 mg/ml BSA and 2 pM 5' ³²P endlabeled aptamer. Assays were conducted as described in the article Methods section using the nitrocellulose filter binding method for measuring binding. A plot of bound aptamer vs. IN concentration was fit to the following equation for ligand binding one-site saturation in SigmaPlot in order to determine the $K_{D,app}$: $y=B_{max}(x)/(K_D+x)$ where x is the concentration of IN and y is the amount of bound aptamer. The above graph is an assay measuring the binding of aptamer IN-1.1 to HIV IN^{F185H/C280S}. The determined $K_{D,app}$ value and the r² value for the fit are shown along with a Table of the data that was plotted. *Counts refer to radioactivity counted bound to nitrocellulose filters that were determined using a scintillation counter.

	P1	P2	P2	P3	P3	P4	P4	P1	
IN-1.1	aaaa <mark>ggtagtg</mark> c	<mark>tgaa</mark> tt	cgU <mark>UUCA</mark>	AGU <mark>GU</mark> AUA	UUAACU	ACGCAUCUUUC	CCCC <mark>UGCGUA</mark> ur	cgcuauc	caguuggccu
(RNA parameters)		(((())))	((()))	(((()))))))))))))	
(DNA parameters)		(((($\dots)))))$		(((((())))))		
IN-1.2	aaaa <mark>ggtagtg</mark> c	<mark>tgaa</mark> tt	.cgU <mark>UUCA</mark> A	AGU <mark>GU</mark> AUA	UUA <mark>AC</mark> U	ACGCAUCUCUC	CCCCUGCGUAut	lcgcuauc	caguuggccu
(RNA parameters)		(((())))	((()))	(((()))))))))))))	
(DNA parameters)		(((($\dots)))))$		(((((())))))		
IN-1.3	aaaa <mark>ggtagtg</mark> c	<mark>tgaa</mark> tt	.cgU <mark>UUCA</mark> A	AGU <mark>GU</mark> AUA	UUA <mark>AC</mark> U	UCGCAUCUUUC	CCCC <mark>UGCGUA</mark> ur	lcgcuauc	caguuggccu
(RNA parameters)	((((((((. (((((()))).))	((()))))))))))
(DNA parameters)	. ((((((((. (((. ((())))))))))))))).	((()))	
IN-1.4	aaaa <mark>ggtagtg</mark> c	<mark>tgaa</mark> tt	.cgU <mark>UUCA</mark> A	AGU <mark>GU</mark> AUA	UUA <mark>AC</mark> U	ACGCAUCUUUC	CCCAUGCGUAut	l <mark>cgcuauc</mark>	caguuggccu
(RNA parameters)		(((())))	((()))	((((())))))))))))))	
(DNA parameters)		(((())))		(((((())))))		
IN-1.5	aaaa <mark>ggtagtg</mark> c	<mark>tgaa</mark> tt	.cgU <mark>UUCA</mark>	AGU <mark>GU</mark> AUA	UUA <mark>UC</mark> U	ACGCAUAUUUC	CCCAUGCGUAut		caguuggccu
(RNA parameters)	(((((((((((())).))))))))))))	((((())))))	. (((())))
(DNA parameters)		(((())))		(((((())))))		
IN-2.1	aaaa <mark>ggtagtg</mark> c	<mark>tgaa</mark> tt	.cgU <mark>UUCA</mark> A	AGU <mark>CC</mark> AUA	UUA <mark>GG</mark> G	UGAGAUAUAAU	CCUCUCUCACut	l <mark>cgcuauc</mark>	caguuggccu
(RNA parameters)		(((())))	(())((((())))))))))))))	
(DNA parameters)		(((())))		((((())))))		
IN-3.1	aaaa <mark>ggtagtg</mark> c	tgaatt	.cgU <mark>AGU</mark> A <mark>l</mark>	<mark>JCA</mark> AGUCC	AUAUUA	GGGUGGGAUC.	AAACCCCACAut	l <mark>cgcuauc</mark>	caguuggccu
(RNA parameters)	((((.((.))).)))))		(((()))))	. (((())))
(DNA parameters)						(((()))))		
IN-3.2	aaaa <mark>ggtagtg</mark> c	tgaatt	.cgU <mark>AGU</mark> Al	<mark>JCA</mark> AGUCC	AUAUUA	. <mark>GG</mark> GGUGGGAUC	AAUUUCCAUAut	l <mark>cgcuauc</mark>	caguuggccu
(RNA parameters)	((((((.))).)))))((()))((((())))))	. (((())))
(DNA parameters)					(((.((((())))))).)))	
IN-3.3	aaaa <mark>ggtagtg</mark> c	tgaatt	.cgU <mark>AGU</mark> AI	<mark>JCA</mark> AGUCC	AUAUUA	GGGUGGGAUC.	AAACUCCACAut	l <mark>cgcuauc</mark>	caguuggccu
(RNA parameters)	((((. (((.))).))))).((.((((((((((())).))))).)))))).))
(DNA parameters)	((. ((((((.((((()))))))	.))(((()))))))))))	
IN-3.4	aaaa <mark>ggtagtg</mark> c	tgaatt	.cgU <mark>AGU</mark> AI	<mark>JCA</mark> AGUCC	ACAUUA	. <mark>GG</mark> GGUGGAAUC	AAACCCCACCut	l <mark>cgcuauc</mark>	caguuggccu
(RNA parameters)	(((((((((()))))))		(((()))))))))))))))))))))))))))))))))))))	. (((())))
(DNA parameters)	(((((.(((. ((. ()	()).)))))	(((()))))))))))))))))))))))))))))))))))))		
IN-4.1	aaaa <mark>ggtagtg</mark> c	tgaatt	.cgAUA <mark>AAI</mark>	JA <mark>UCA</mark> AGA	CCAUAU	IUA <mark>GG</mark> UGGGGAG	AUUGAUCCCAut	l <mark>cgcuauc</mark>	caguuggccu
(RNA parameters)	((((((((.((.	. (((. ((((((((())))	.)))))))))).))))))))
(DNA parameters)		. ((((.	. (((. (((.((((())))).))).))))))))))))))))))))))))))))))))))))))	
IN-4.2	aaaa <mark>ggtagtg</mark> c	tgaatt	cgUA <mark>AAU</mark> A	A <mark>UCA</mark> AGUC	CAUAUU	IA <mark>GA</mark> UGGGGAGA	UAAUACCCAUur	lcgcuauc	caguuggccu
(RNA parameters)	((((.((.)))))))).((.(((.((. ((((((((($(\ldots \ldots)))))\\$).)))))	.))))))))))))))))))))))))))))))))))))))
(DNA parameters)		(((((()))	.))))))))		
IN-5.1	aaaa <mark>ggtagtg</mark> c	<mark>tgaa</mark> tt	cgAUUGAA	AU <mark>UUCA</mark> AG	gu <mark>ca</mark> aua	UUA <mark>UG</mark> CCGGAC	UAUAUUCCGGut	lcgcuauc	caguuggccu
(RNA parameters)	((((((((.((.	. (((((())))).	(((((()))))).)))).))))))))
(DNA parameters)		((((()))))))))))		
IN-6.1	aaaa <mark>ggtagtg</mark> c	tgaatt	.cgAUGG <mark>U(</mark>	CAAAUUCA	AGUCCA	UAUUA <mark>GG</mark> CAGG	CAUCAU <mark>CCUG</mark> ur	l <mark>cgcuauc</mark>	caguuggccu
(RNA parameters)		((. (((((())))))	.))))))))))))))
(DNA parameters)		((((())))))		((((())))).		
IN-7.1	aaaa <mark>ggtagtg</mark> c	tgaatt	cgGA <mark>AAU</mark> A	A <mark>UUA</mark> AGUC	CUAUU	A <mark>GG</mark> GGGGGAAA	AUAAUACCCAur	lcgcuauc	caguuggccu
(RNA parameters)	((((((((. (((((())))))	.))))))))))))))
(DNA parameters)		(((()	())))))))))).		

Figure S2. Predicted secondary structures of the round 10 sequences that conform to the four-way junction structural motif. All sequences from the lineages 1 - 7 are shown. The four final predicted base-pairing elements are highlighted, color coded, and labeled. Secondary structures predicted for individual sequences by RNAfold using default RNA parameters (RNA parameters) and those predicted using DNA energy parameters (DNA parameters) are shown in standard bracket notation.

		P1	P2	P2	P3	P3 P4	P4	P1		
IN-1.1	aaaa	<mark>ggtagtg</mark> c	<mark>tgaa</mark> ttcgl	J <mark>UUCA</mark> AGU	GUAUAUUA	ACUACGCAU	UCUUUCCCCC <mark>UGCGUA</mark>	uu <mark>cgcuauc</mark> cagu-uggccu	51 pM	
IN-1.1(-10)			<mark></mark>	<mark></mark>				<mark></mark>	88 pM	
IN-1.1(flip)		.catc	<mark></mark>	<mark></mark>				<mark>gaug.</mark>	89 pM	
IN-1.1(U61C,U62C)								cc	92 nM	
IN-1.1(U34C,U35C)					CC				1.6 μ M	
IN-1.1(dis-P3L3)				C	.CG	CG			1.7 μ M	
IN-1.1(delta-L3)				C	.CG	CACG			1.8 μ M	
IN-1.1(U28C,U61C,U62A)				C				ca	2.6 µM	
IN-1.1(U28C,dis-P1)				C				cacta	>4 μ M	
A-disrupt-all			aaaaA	AA			АААААААААА	a.aa	>4 μ M	
		P1	P2		P2	P3	P3 P4	P4 P1		
IN-2.1	aaaa	ggtagtgc	tgaattcg-		U <mark>UUCA</mark> AGU	JCCAUAUUA	<mark>GG</mark> GUGAGAUAUAAUCC	UCUCUCACuu <mark>cgcuauc</mark> cag	uuggccu 44	рМ
IN-2.1(flip)		.catc	<mark></mark>		• • • • • • • • •		<mark></mark>		101	рМ
IN-2.1(-10)			<mark></mark> [.]				<mark></mark>	· · · · · · · · · · · · · · · · · · ·	106	рМ
IN-(6.1/2.1)				UGG <mark>UCAA</mark>	A			· · · · · · · · · · · · · · · · · · ·	118	рМ
IN-(6.1/2.1)(-10)				UGG <mark>UCAA</mark>	A		<mark></mark>	· · · · · · · · · · · · · · · · · · ·	146	рМ
IN-2.1(altP1)	• • • •	.ca			••••				ug 8.9	nM

Figure S3. Sequences variants based on IN-1.1 and IN-1.2. The sequences are shown relative to IN-1.1 and IN-2.1. For, sequences predicted to adopt the secondary structure depicted in Figure 2 the base-pairing elements are highlighted, color coded, and labeled. All other sequences are predicted to adopt alternative structures. The $K_{D,App}$ for each construct is indicated to the right of the sequence.



Figure S4. Predicted base-pairing probabilities for sequence variants based on IN-1.1 Dot plots containing the base-pair probabilities predicted by RNAfold are shown for the sequences indicated above the plot. The squares above the diagonal represent the base-pair probabilities. Above the diagonal the size of the squares is proportional to the probability of the base-pair. The squares below the diagonal represent the base-pairs present in the predicted minimum free energy structure. The positions in the plot that correspond to P1 and P2 are circled and labeled.



Figure S5. Predicted base-pairing probabilities for sequence variants based on IN-2.1 Dot plots containing the base-pair probabilities predicted by RNAfold are shown for the sequences indicated above the plot. The squares above the diagonal represent the base-pair probabilities. Above the diagonal the size of the squares is proportional to the probability of the base-pair. The squares below the diagonal represent the base-pairs present in the predicted minimum free energy structure. The positions in the plot that correspond to P1 and P2 are circled and labeled.