Supplemental Material:

Probing Anomalous Structural Features in Polypurine tract-containing RNA:DNA Hybrids with Neomycin B

Robert G. Brinson,[1,3†] Kevin B. Turner,[2†] Hye Young Yi-Brunozzi,[3] Stuart F.J. Le Grice,[3] and Daniele Fabris,[2*] John P. Marino[1]*

[1]Center for Advanced Research in Biotechnology of the University of Maryland Biotechnology Institute and the National Institute of Standards and Technology, Rockville, MD

[2]University of Maryland Baltimore County, Baltimore, MD

[3]HIV Drug Resistance Program, NCI, National Institutes of Health, Frederick, MD

Supplemental Table 1:

Ligand	PPT_{WT}	PPT_{SWP}	PPT_{RNA}	PPT_{DNA}	Relative Affinity
Mitoxantrone	3	3	3	3	DNA>WT≈Swp≈RNA
Ethidium Bromide	3	3	3	3	DNA>WT≈Swp≈RNA
Distamycin	1	1	1	2	DNA>WT>Swp>RNA
Neomycin B	2	1	1	1	WT>Swp>RNA>DNA

Supplemental Table 1: Summary of the stoichiometries and relative binding affinities obtained by titrating an equimolar mixture of the four PPT substrates with increasing amounts of each individual ligand. The reported stoichiometry corresponds to the maximum number of units observed upon 10-fold addition of ligand over the total amount of each duplex substrate in solution (see *Experimental Procedures*).

Supplemental Table 2: Perturbations in chemical shifts assigned to the base proton of the A) PPT_{WT}, B) PPT_{RNA}, C) PPT_{swp} hybrid samples due to the addition and binding of 2 equivalents of NB.

A	Ty3 Wild-Type PPT			2.0 eq. Neomycin			Change in Chemical Shift		
Strand 1	H42	H41	H2	H42	H41	H2	Δ(H42)	Δ(H41)	Δ(H2)
a1			NA			NA			NA
a2			7.73			*			*
c3	8.06	6.66		8.09	6.64		-0.03	0.02	
c4	8.08	6.61		8.11	6.59		-0.03	0.01	
c5	8.04	6.71		8.06	6.67		-0.02	0.03	
a8			7.18			7.13			**0.05**
a10			7.22			7.20			0.02
a12			7.20			7.20			0.00
a14			7.26			7.23			0.03
a17			6.97			6.96			0.01
a18			7.36			7.30			**0.07**
a20			NA			NA			NA
Strand 2	H42	H41	H2	H42	H41	H2	Δ(H42)	Δ(H41)	Δ(H2)
A15			6.77			6.66			**0.11**
C14	7.95	6.56		7.95	6.53		0.00	0.03	
C12	8.08	6.55		8.08	6.52		-0.01	0.03	
C10	8.08	6.55		8.08	6.52		0.00	**0.04**	
C8	8.08	6.55		8.08	6.52		0.00	**0.04**	
C6	8.14	6.55		8.14	6.53		-0.01	0.02	
C5	8.04	6.55		8.06	6.47		-0.02	**0.08**	
C2	8.34	6.88		*	*	*	*	*	*

* exchange broadened; NA - not assigned

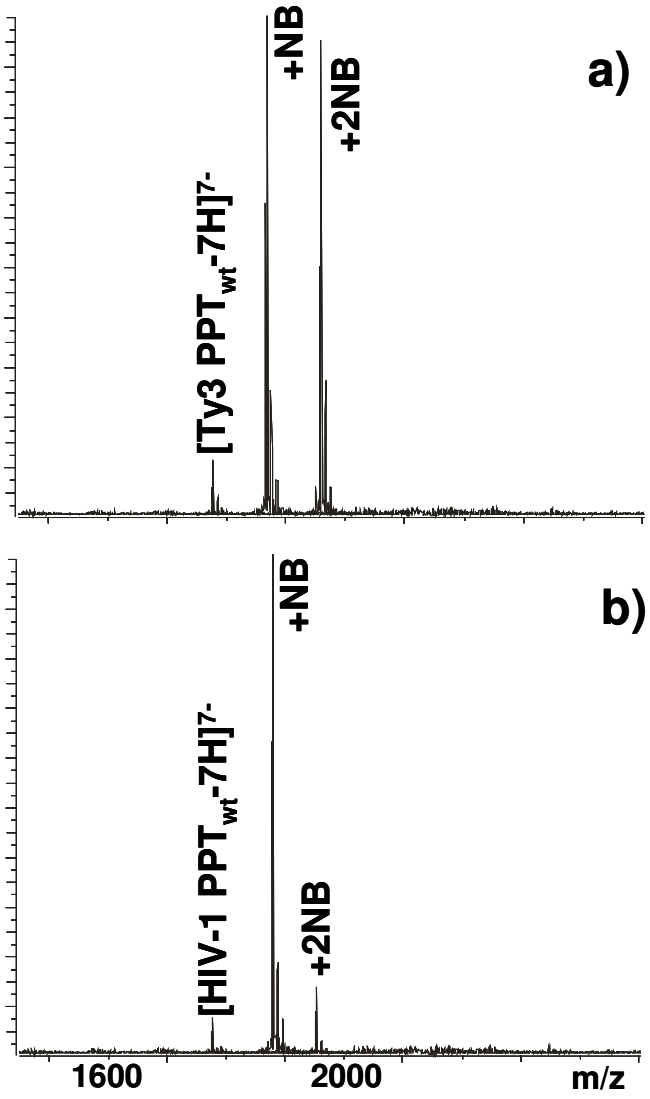
B	Ty3 RNA Duplex			2.0 eq. Neomycin			Change in Chemical Shift		
Strand 1	H42	H41	H2	H42	H41	H2	Δ(H42)	Δ(H41)	Δ(H2)
a1			NA			NA			NA
a2			7.50			*			*
c3	8.234	6.761		*	*		*	*	
c4	8.245	6.635		8.249	6.647		0.00	-0.01	
c5	8.111	6.635		8.113	6.647		0.00	-0.01	
a8			7.00			6.60			**0.41**
a10			7.13			7.16			-0.03
a12			7.13			7.16			-0.03
a14			7.19			7.10			**0.10**
a17			6.86			6.90			-0.04
a18			7.21			7.21			0.01
a20			NA			NA			NA
Strand 2	H42	H41	H2	H42	H41	H2	Δ(H42)	Δ(H41)	Δ(H2)
a15			6.60			6.55			**0.04**
c14	7.943	6.668		7.941	*		0.00		
c12	8.06	6.745		8.058	6.715		0.00	0.03	
c10	8.03	6.745		8.096	6.715		**-0.07**	0.03	
c8	8.097	6.745		8.145	6.715		**-0.05**	0.03	
c6	8.154	6.86		8.157	6.83		0.00	0.03	
c5	8.152	6.647		8.157	6.649		-0.01	0.00	
c2	8.281	6.866		8.293	6.904		-0.01	-0.04	

* exchange broadened; NA - not assigned

C	Ty3 Swapped Duplex			2.0 eq. Neomycin			Change in Chemical Shift		
Strand 1	H42	H41	H2	H42	H41	H2	Δ(H42)	Δ(H41)	Δ(H2)
A1			NA			NA			NA
A2			7.70			*			*
C3	8.06	6.53		8.045	6.46		0.02	0.07	
C4	8.25	6.59		8.251	6.553		0.00	0.04	
C5	8.18	6.59		8.12	6.574		0.06	0.01	
A8			7.00			6.968			0.03
A10			7.09			7.079			0.01
A12			7.09			7.079			0.01
A14			7.15			7.138			0.01
A17			6.95			6.958			-0.01
A18			7.11			7.143			**-0.04**
A20			NA			NA			NA
Strand 2	H42	H41	H2	H42	H41	H2	Δ(H42)	Δ(H41)	Δ(H2)
a15			6.82			6.728			**0.09**
c14	8.20	6.77		8.112	6.744		0.08	0.03	
c12	8.21	6.84		8.156	6.745		0.05	**0.09**	
c10	8.21	6.84		8.156	6.745		0.05	**0.09**	
c8	8.21	6.84		8.156	6.745		0.05	**0.09**	
c6	8.22	6.82		8.267	6.726		-0.04	**0.09**	
c5	8.32	6.81		8.408	6.702		-0.09	0.11	
c2	8.12	6.95		8.205	6.929		-0.09	0.02	

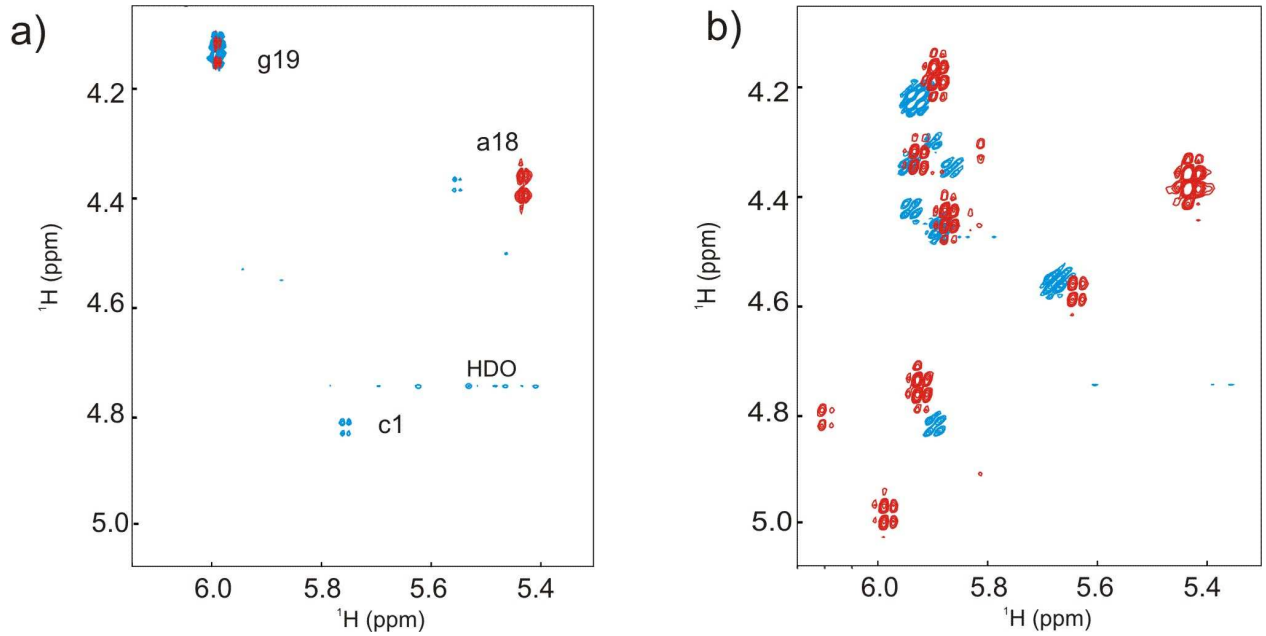
* exchange broadened; NA - not assigned

Supplemental Figure 1:



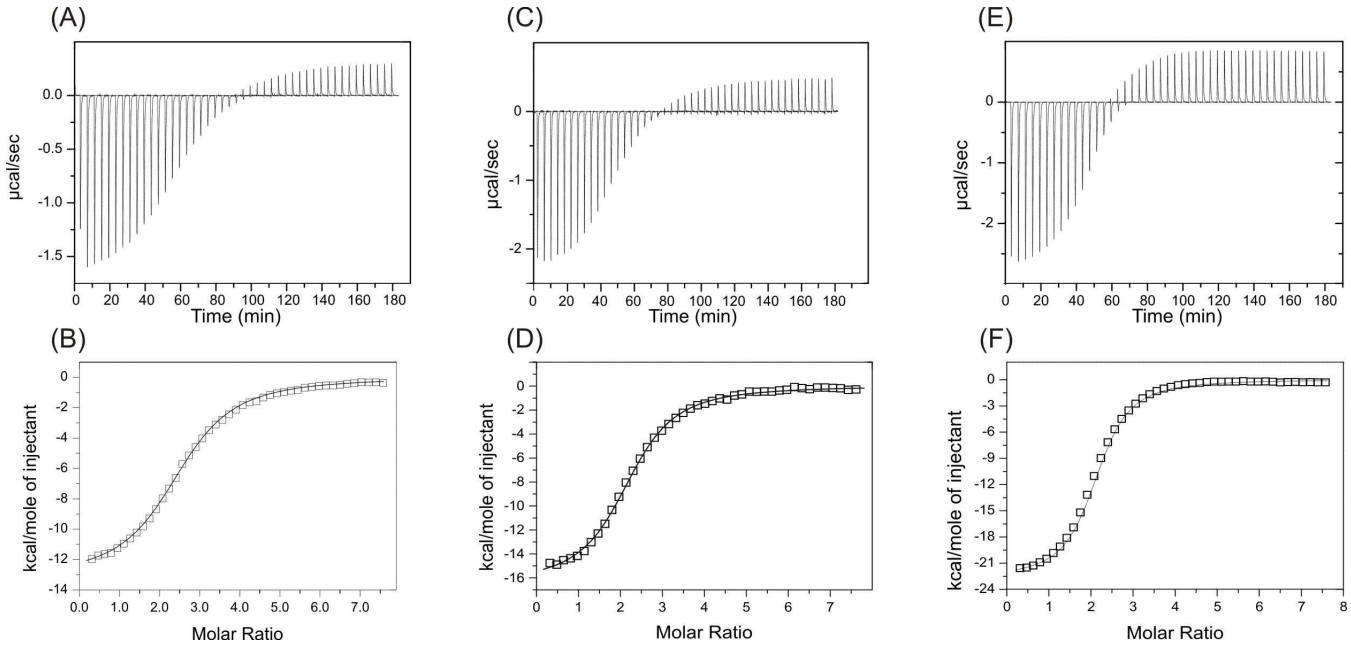
Supplemental Figure 1: Nanospray-FTICR mass spectra of: a) 5 μM $Ty3_{wt}$ PPT hybrid and b) 5 μM HIV-1_{wt} PPT hybrid in the presence of a 10-fold excess of NB.

Supplemental Figure 2:



Supplemental Figure 2: An overlay of the expanded H1'-H2' region of a 2D DQF-COSY experiment applied to the Ty3 A) PPT_{WT} and B) PPT_{swp} hybrids in the absence (red) and presence (blue) of NB. Chemical shift perturbations result from NB binding.

Supplemental Figure 3:



Supplemental Figure 3: ITC titration of selected PPTs with NB in 80 NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 at pH 7.0 and 40 ^oC. A) ITC profile of PPT_{WT}; B) Integration of panel a) with curve-fit.; C) ITC profile of PPT_{swp}; D) Integration of panel C) with curve-fit; E) ITC profile of PPT_{swp}; F) Integration of panel E) with curve-fit. The integrations in panels B), D), F) were corrected for the heats of dilution from the titration of NB into the buffer alone. All curve-fitting employed a model of one set of equivalent sites.