SUPPLEMENTARY DATA TO

Dynamic interactions of the HIV-1 Tat with nucleic acids are critical for Tat activity in reverse transcription

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Time-resolved fluorescence measurements

Time-resolved fluorescence measurements were performed using the time-correlated single-photon counting technique, as described elsewhere.(61,85). Briefly, excitation pulses at 315 nm were generated by a pulse-picked frequency-tripled titanium-sapphire laser (Tsunami, Spectra Physics) pumped by a diode-pumped solid-state Nd: YVO_4 doubled laser (Millennia X, Spectra Physics). Emission was collected at 370 nm through a polarizer set at the magic angle and a 8-nm band-pass monochromator (H10, Jobin-Yvon). The single-photon events were detected with a micro-channel plate (R3809U, Hamamatsu) photomultiplier coupled to a pulse pre-amplifier (HFAC, Becker and Hickl GmbH) and recorded on a time-correlated single photon counting board (SPC-130, Becker and Hickl GmbH). The full-width at half maximum of the instrumental response function was ~ 50 ps. Measured fluorescence decays were deconvoluted with the instrumental response function and fitted to retrieve the most probable lifetime distribution using the maximum entropy method.(86,87) The mean lifetime $\langle\tau\rangle$ was calculated according to $\langle\tau\rangle = \Sigma\alpha_i\tau_i$. The population α_0 of dark species was calculated by equation 1, *while the* remaining amplitudes of the fluorescent populations were recalculated according to $\alpha_{ic} = \alpha_i \times (1 - \alpha_o)$. All fitting procedures were carried out with the maximum entropy method Pulse5 software.

Gel-shift annealing assays

The heat-denatured control (0.1 pmol of the 5'-end labelled dTAR DNA at 2×10^5 cpm/pmol or 0.1 pmol of the 5'-end labelled TAR RNA at 2×10^5 cpm/pmol in 10 µL of double-distilled water) was performed by heating at 90 °C for 2 min and chilling for 2 min on ice, and mixing with 2.5 µL of loading buffer (50 % w/v glycerol, 0.05 % w/v bromophenol blue, 0.05 % w/v xylene cyanol). The annealing assays were carried out in a final volume of 10 µL. The 5'-end labelled dTAR DNA (0.1 pmol at 2×10^5 cpm/pmol) or the 5'-end labelled TAR RNA (0.1 pmol at 2×10^5 cpm/pmol) in 3.6 µL of water was heated at 90 °C for 2 min and chilled for 2 min on ice. Then, 0.9 µL of renaturation buffer was added (final concentrations: 30 mM NaCl, 0.2 mM $MgCl_2$ and 25 mM Tris-HCl pH 7.5) and the sample was incubated at 20°C for 10 min. Unlabeled cTAR DNA (1 pmol) underwent the same renaturation treatment and was then added to refolded dTAR ^{32}P-DNA or TAR ^{32}P-RNA. The reaction mixture was then incubated at 20°C for various times in the absence or presence of peptide (3.3 pmol). Reactions were stopped by adding SDS to a final concentration of 0.2%. Peptides were digested by adding proteinase K to a final concentration of 0.25 mg/mL and incubated at 20°C for 30 min. At the end of incubation time, 3.5 µL of loading buffer was added to the assays. The samples were analyzed by electrophoresis on a 12 % polyacrylamide gel (37.5:1 (w/w), acrylamide/bisacrylamide) at 25 °C in 1 X TBE buffer (90 mM Tris-borate (pH 8.3), 2 mM EDTA). After electrophoresis, the gel was fixed, dried and autoradiographed

Supplementary References

85. Godet,J., Ramalanjaona,N., Sharma,K.K., Richert,L., de Rocquigny,H., Darlix,J.-L., Duportail,G. and Mély,Y. (2011) Specific implications of the HIV-1 nucleocapsid zinc fingers in the annealing of the primer binding site complementary sequences during the obligatory plus strand transfer. *Nucleic Acids Res.*, **39**, 6633–6645.

86. Livesey,A.K. and Brochon,J.C. (1987) Analyzing the distribution of decay constants in pulse-fluorimetry using the maximum entropy method. *Biophys. J.*, **52**, 693–706.

87. Brochon,J.C. (1994) Maximum entropy method of data analysis in time-resolved spectroscopy. *Meth. Enzymol.*, **240**, 262–311.

Table S1

Time-resolved fluorescence parameters of Tat(44-61)Y47W in its free and cTAR-bound form

	α_0[a]	τ_1(ns)[a]	α_1[a]	τ_2 (ns)[a]	α_2[a]	τ_3 (ns)[a]	α_3[a]	$<\tau>$ (ns)[a]	Φ[a]
Tat(44-61)Y47W	--	3.1 ± 0.1	0.64	1.1 ± 0.06	0.36	--	--	2.36	0.105 ± 0.003
Tat(44-61)Y47W/ cTAR	0.46	2.8 ± 0.2	0.21	0.83 ± 0.04	0.19	0.10 ± 0.01	0.14	1.37	0.033 ± 0.002

[a]α_0, $\tau_{1\text{-}3}$, $\alpha_{1\text{-}3}$, $<\tau>$ and Φ designate the fraction of dark species, the fluorescence lifetimes and their relative amplitudes, the mean lifetime and the quantum yield, respectively. Excitation and emission wavelengths were 295 and 350 nm, respectively. Experiments were performed at 20 °C in 25 mM Tris pH 7.5 containing 30 mM NaCl and 0.2 mM $MgCl_2$

Table S2

Activation energy and transition state enthalpy for cTAR / dTAR annealing reactions promoted by Tat(44-61) derivatives.

peptide	$E_{a1,\,2}$ (kcal.mol^{-1})[a]	$\Delta H^{\ddagger}_{1,\,2}$ (kcal.mol^{-1})[b]
Tat(44-61)	9.6 ± 0.6	9.0
	18 ± 2	17.4
Tat(50-61)	9 ± 1	8.5
	20 ± 2	19.6
Tat(44-57)	9.5 ± 0.6	9.0
	18 ± 2	17.5
Tat(44-61)R52A,R53A	11 ± 2	10.3
	23.5 ± 3	22.9
Tat(44-61)R55A,R56A	9.3 ± 0.7	8.7
	20.8 ± 2	20.2
Tat(44-61)$_{ac}$	12 ± 3	11.7
	21.0 ± 0.5	20.4
Tat(44-61)Y47A	13.4 ± 0.7	12.8
	18 ± 2	17.8
Tat(44-61)Q54A	10 ± 2	9.1
	17 ± 2	16.4

[a]determined from the fits of the data of Figure 7 to equation 5.

[b]calculated using $\Delta H^{\ddagger} = E_a - RT$ with T = 293 K

FIGURES LEGENDS

Supplementary Figure S1. Representative titration curves of cTAR by Tat(44-61) derivatives. Titrations were performed by adding increasing amounts of Tat(44-57) (○), Tat(44-61)$Q_{54}A$ (■) or Tat(44-61)ac (□) to 10 nM cTAR-3'-Fl. Complex formation was followed by measuring the increase in the steady-state fluorescence anisotropy. The solid lines corresponds to the fit of the binding data with equation 6 and the K_{obs} values of Table 1.

Supplementary Figure S2. Effect of Tat(44-61) on the quantum yields of 2-Ap-labeled cTAR sequences. The quantum yield values of 5 µM cTAR sequences labeled by 2-aminopurine (2-Ap) at the positions indicated on the X-axis were determined at 20°C in the presence of 3 equivalents of Tat(44-61). The results were expressed as the percentage of increase of the quantum yield, induced by the interaction with Tat(44-61). The strongest effects were observed for positions 9 and 49. Quantum yield was calculated using free 2Ap as a reference [0.68[54]], with an excitation wavelength of 315 nm. Error bars on quantum yields are about 10%.

Supplementary Figure S3. Gel-shift analysis of cTAR/dTAR annealing promoted by Tat(44-61) derivatives. Annealing assays were performed as described in Materials and Methods. Lane C1, dTAR ^{32}P-DNA was incubated at 20°C for 240 min in the absence of peptide. dTAR ^{32}P-DNA mixed with cTAR DNA was incubated in the absence or the presence of peptide at 20°C for 5, 15, 30, 60, 120, 180 and 240 min. Monomeric form of dTAR DNA is indicated by dTAR*. The cTAR/dTAR duplex is indicated by dTAR*/cTAR.

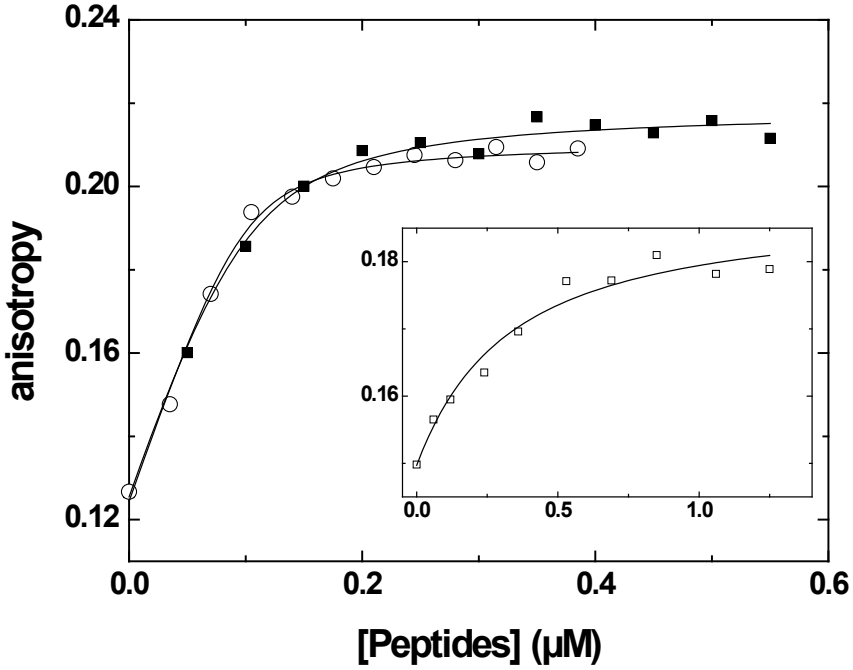
0.24
0.20
0.16
0.12
anisotropy
0.0
0.2
0.4
0.6
[Peptides] (μM)
0.18
0.16
0.0
0.5
1.0

Figure S1

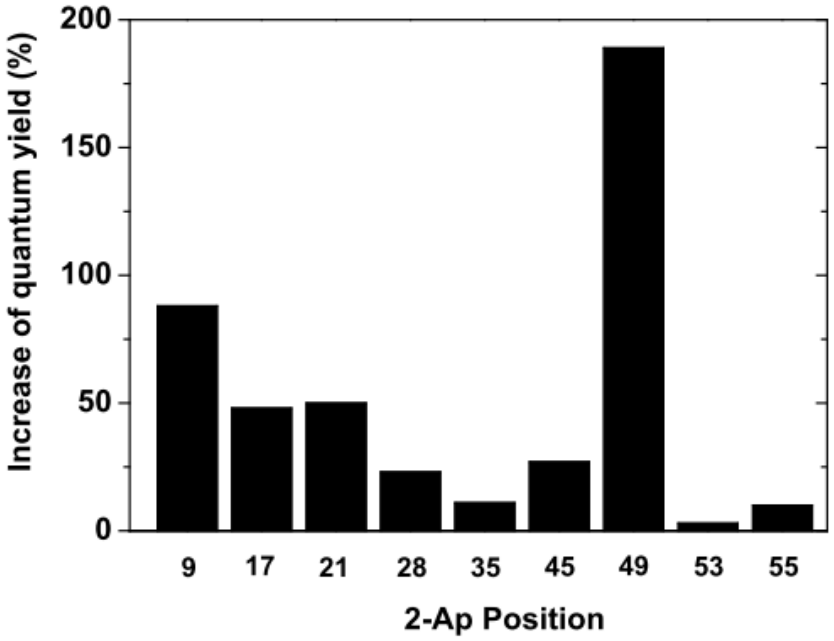
Increase of quantum yield (%)
200
150
100
50
0
9
17
21
28
35
45
49
53
55
2-Ap Position

Figure S2

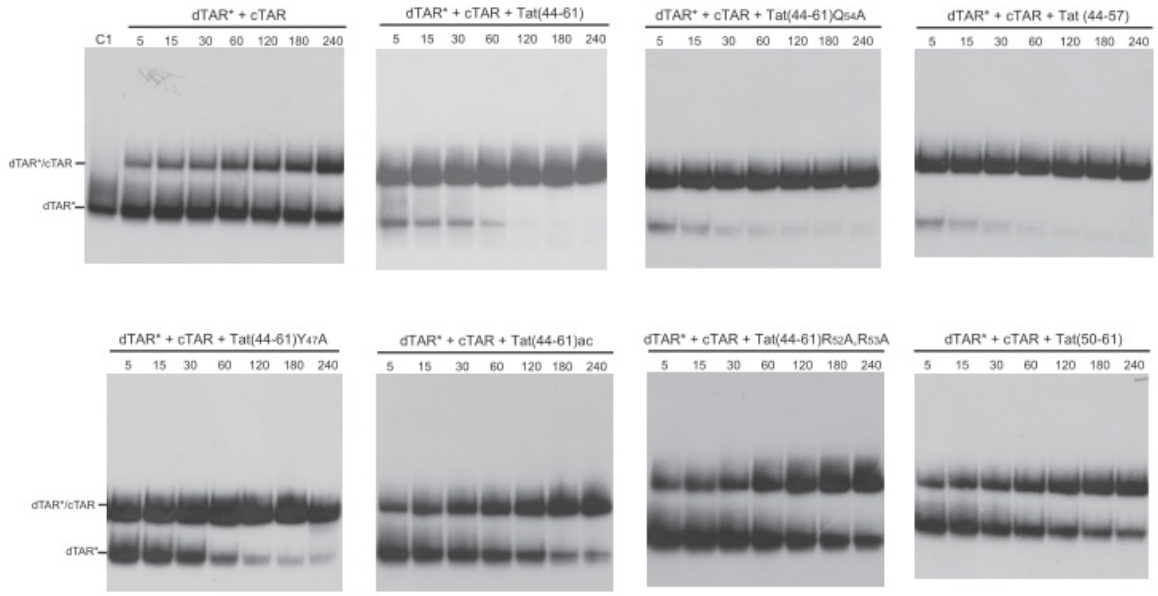
dTAR* + cTAR
C1
5 15 30 60 120 180 240
dTAR* + cTAR + Tat(44-61)
dTAR* + cTAR + Tat(44-61)Q54A
dTAR* + cTAR + Tat (44-57)
dTAR*/cTAR
dTAR*
dTAR* + cTAR + Tat(44-61)Y47A
dTAR* + cTAR + Tat(44-61)ac
dTAR* + cTAR + Tat(44-61)R52A,R53A
dTAR* + cTAR + Tat(50-61)

Figure S3