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Supplementary Materials

The Peptide TAT-I24 with Antiviral Activity against DNA Viruses Binds Double-Stranded DNA with High Affinity

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Figure S1

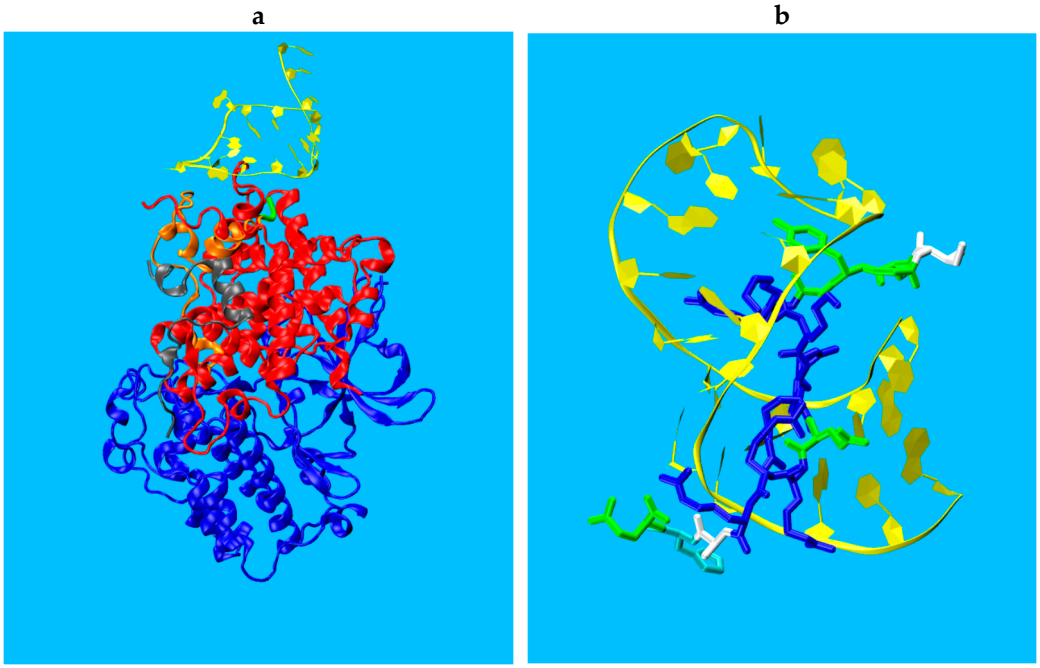

Figure S1. Structural information of the TAT protein as template for affinity studies. (**a**), the peptide sequence GRKKRRQRRR is found in the TAT-protein of HIV-1 (1). A crystal structure (pdb code 6CYT) identifies its location in close proximity to the RNA stem loop (glycine (G) indicated in green, the RNA in yellow). Backbone interactions between R/K and the phosphate groups of the RNA are likely (2). (**b**) Close-up of the complex as revealed by the NMR structure (pdb code 6MCE) with again pronounced R/K-backbone interactions (3).This structure may serve as template for systematic affinity studies of DNA/peptide samples.

Figure S2

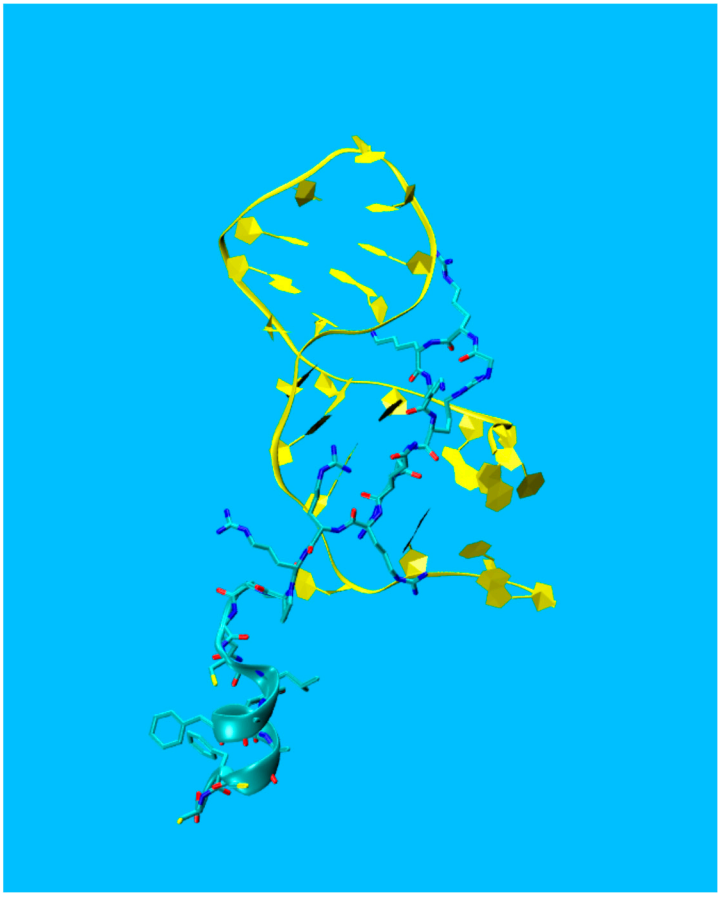

Figure S2. Structural sample, TAT-I24/ds(AT). The assembled complex was subjected to MM-PBSA analysis to estimate binding affinities (4, 5) of TAT-like peptides to DNA (TAT-I24 shown in cyan/blue/red, ds(AT) in yellow). DNA docosamers (6) are considered with sequences ds(AT):CATATATATCCCCATATATATG and ds(GC):CGCGCGCGCTTTTGCGCGCGCG where the underlined stretch of nucleotides supposedly forms the hairpin loop region (yellow tip at the top). Two peptides are taken into account, GRKKRRQRRRPPQ (TAT (48-60)), and GRK-KRRQRRRPPQCLAFYACFC (TAT-I24) with I24 in likely helical conformation (as observed in long-term MD simulations; see lower left corner).

Table S1. Binding affinities of TAT or TAT-I24.

Binding affinities were determined from MM-PBSA analysis (4, 5) of always 250 ns of MD-simulation in explicit water (AMBER18, pmemd.cuda, NpT, 300 K,1 bar, Δt = 1 fs, protein.ff14SB, DNA.bsc1, water.tip3p, ionsjc tip3). ΔG values given in parentheses include the approximation of entropic contributions.

System	MD time considered [ns]	$\Delta G^{binding}_{GB}$ [kcal/mol]	$\Delta G^{binding}_{PB}$ [kcal/mol]
ds(AT)/TAT	entire 250	−116.9 (132.1)	−123.9 (125.2)
	final 200	−118.4 (114.7)	−124.3 (108.8)
	final 150	−121.2 (86.0)	−127.4 (79.8)
	final 100	−119.4 (56.5)	−127.6 (48.4)
	final 50	−107.6 (18.3)	−118.2 (7.7)
ds(AT)/TAT −I24	entire 250	−124.6 (171.8)	−129.1 (167.3)
	final 200	−124.3 (145.6)	−128.9 (141.0)
	final 150	−126.5 (109.2)	−129.6 (106.1)
	final 100	−133.0 (53.5)	−133.3 (53.2)
	final 50	−144.9 (−20.6)	−139.2 (−15.0)
ds(GC)/TAT	entire 250	−113.1 (112.1)	−120.4 (104.7)
	final 200	−117.9 (92.5)	−124.3 (86.1)
	final 150	−121.5 (70.5)	−127.4 (64.6)
	final 100	−122.8 (41.7)	−128.4 (36.2)
	final 50	−125.8 (−8.1)	−131.0 (−13.2)
ds(GC)/TAT-I24	entire 250	−170.7 (114.0)	−164.3 (120.4)
	final 200	−171.0 (88.4)	−164.6 (94.8)
	final 150	−171.0 (58.1)	−164.7 (64.4)
	final 100	−167.9 (20.0)	−162.7 (25.1)
	final 50	−165.0 (−41.2)	−159.8 (−36.0)

i) GB solvation models should be identical to PB with reliability (PB > GB)

ii) TΔS terms show strong variation with increasing MD time hinting at substantial flexibility of the peptide

iii) ΔG results neglecting the entropy seem to converge rapidly

iv) The underlined result is a suggested compromise for direct qualitative/semi-quantitative comparison

v) Ranking w.r.2 binding affinity, ds(GC)/TAT-I24 > ds(AT)/TAT-I24 > ds(GC)/TAT ≈ ds(AT)/TAT

vi) For theoretical background of MM/PBSA analysis please see Kollman et al. (5)

Video S1 (dna-tat-md-250ns.avi).

Conformational dynamics of TAT-I24 DNA complexes.

This movie compares the conformational dynamics of all 4 complexes studied in aqueous solution as observed during 250 ns of MD simulation. The time course of the MD trajectories is represented as 360° views.

Figure S3

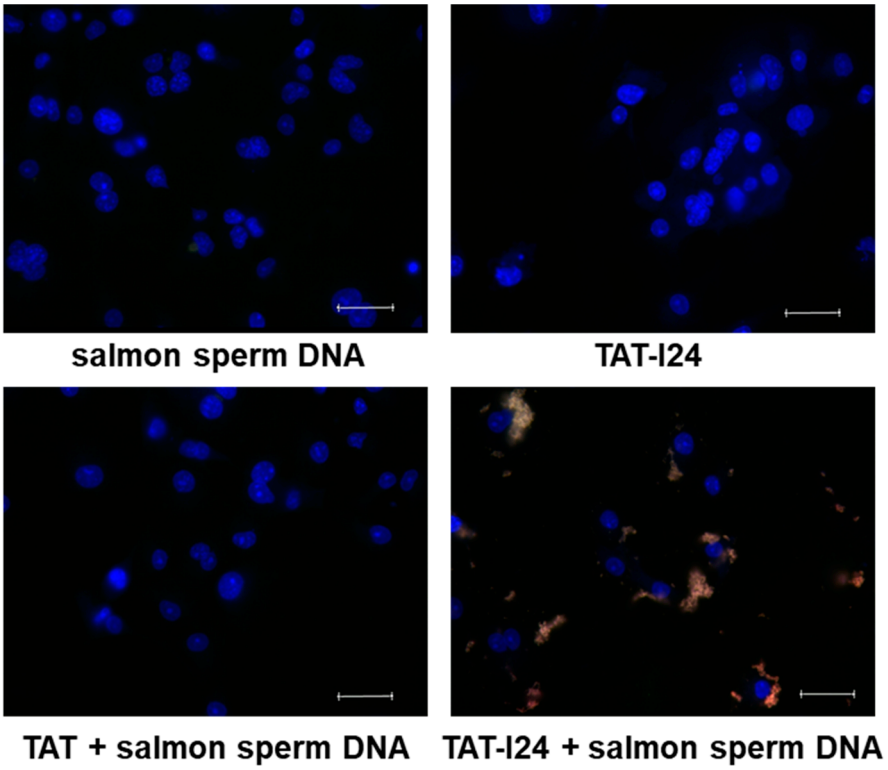

Figure S3. Fluorescence emission of a complex of TAT-I24 with DNA. COS-7 cells were incubated with 1 mg/ml salmon sperm DNA (a), 20 μM TAT-I24 (b), 20 μM TAT (48-60) and 1 mg/ml salmon sperm DNA (c) or 20 μM TAT-I24 and 1 mg/ml salmon sperm DNA (d) for one hour before fixation and staining with DAPI. Images shown represent an overlay of the channels blue (DAPI), green and red (40x objective). Bars indicate 40 μM.

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