Effects of the Nature and Concentration of Salt on the Interaction of the HIV-1 Nucleocapsid Protein with SL3 RNA

SUPPLEMENTARY MATERIAL

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Caption for Figure S1.

Ideal titrations upon adding nucleic acid to protein "AddNA" at values of K_d indicated near each curve. The total RNA concentration, $\boldsymbol{R_t}$, increases from left to right along horizontal axis; $\boldsymbol{R_t/P_t}$ is the mole ratio of total RNA over total protein (*27, 32*). The vertical axis gives the W37 fluorescence intensity relative to the initial intensity, and assumes zero fluorescence when the protein is fully saturated with RNA. Titrations were calculated for 1:1 complexes at constant $\boldsymbol{P_t}$ = 0.3 μM. Dashed straight lines show titrations of complexes in the limit where binding constants approach infinity ($K_d \rightarrow 0$) for 1:1 (R_1P_1) and 1:2 (R_1P_2).

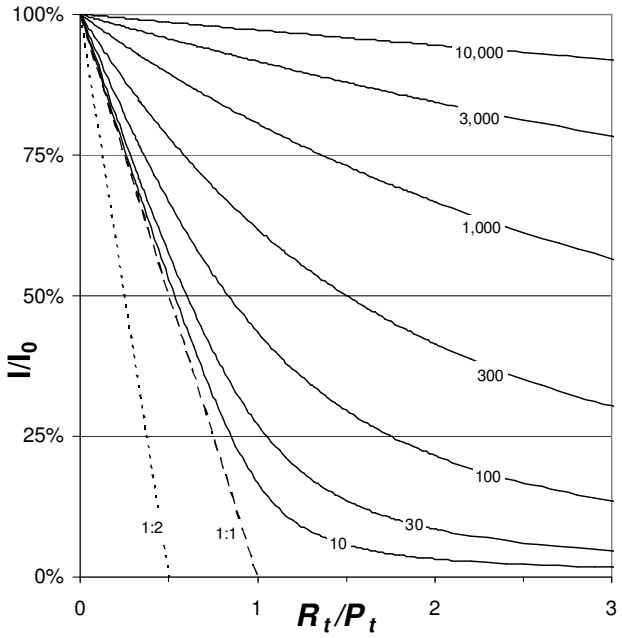

Figure S1.

Caption for Figure S2.

Ideal titrations upon adding adding protein to nucleic acid "AddPro" at values of K_d indicated near each curve. Titrations were calculated for 1:1 complexes at constant $\boldsymbol{R_t}$ = 0.3 μM. They are plotted as relative W37 fluorescence intensity (arbitrary units) vs. $\boldsymbol{P_t/R_t}$, with the protein concentration increasing left to right. The meaning of the 1:1 and 1:2 lines is the same as in Fig. S1. If $\boldsymbol{P_t}$ is increased in the absence of RNA, the intensity will follow the straight line for the "No Binding" limit ($K_d^{-1} \rightarrow 0$).

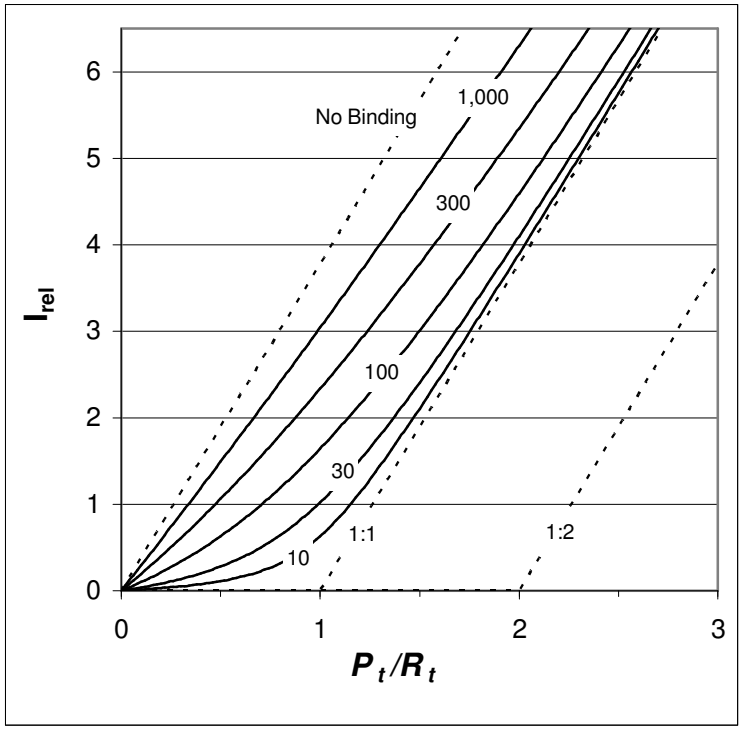

Figure S2.

Caption for Figure S3.

AddNA assays at [NaCl] = 0.050 M measured on different days as in Fig. 4 (main text). The limit lines are for R_1P_1 (long dashes) and R_1P_2 (short dashes; this line intersects the x-axis at the mole ratio, R_t/P_t = 1/2). Solid diamonds show 0.200 M NaCl data identical to that in Fig. 4a (main text).

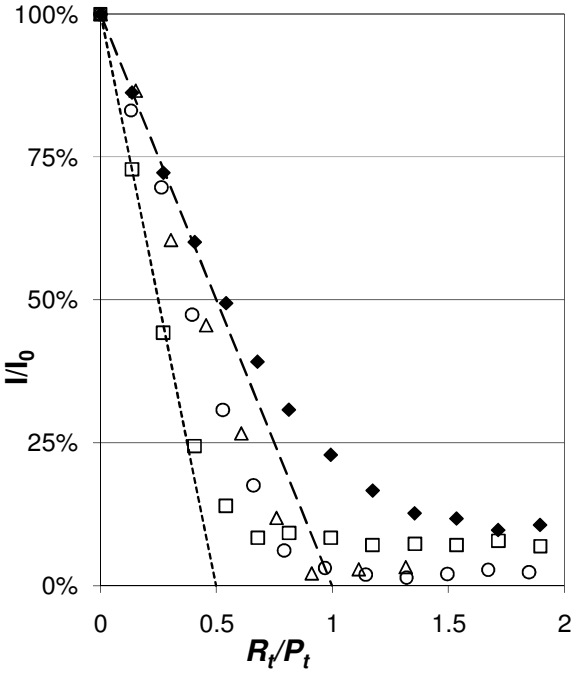

Figure S3

Caption for Figure S4.

AddPro titrations at very low [NaCl]. Four data sets were measured at 0.01 M NaCl (open symbols) and at one at 0.025 M (closed triangles) on different days and with different protein preparations ($\boldsymbol{R_t}$ = 0.075 μM). The solid limit line (marked 0:1) occurs when $\boldsymbol{R_t} = 0$ and $\boldsymbol{P_t}$ is increased. The best fit slope for each 0:1 line was determined by linear regression (setting the intercept = 0). All five data sets were normalized to the same slope for the 0:1 line and to assign relative fluorescence $I_{rel} = 100$ for the data point with the highest intensity; the normalizing factor for a particular 0:1 data set was applied to the data with $\boldsymbol{R_t}$ = 0.075 μM data collected on the same day. The normalized data points for the 0:1 data are shown by small symbols that match those for the corresponding $\boldsymbol{R_t}$ = 0.075 μM data. The standard deviation from the normalized 0:1 line for the 60 data points with $\boldsymbol{R_t} = 0$ was 1.3 relative fluorescence units.

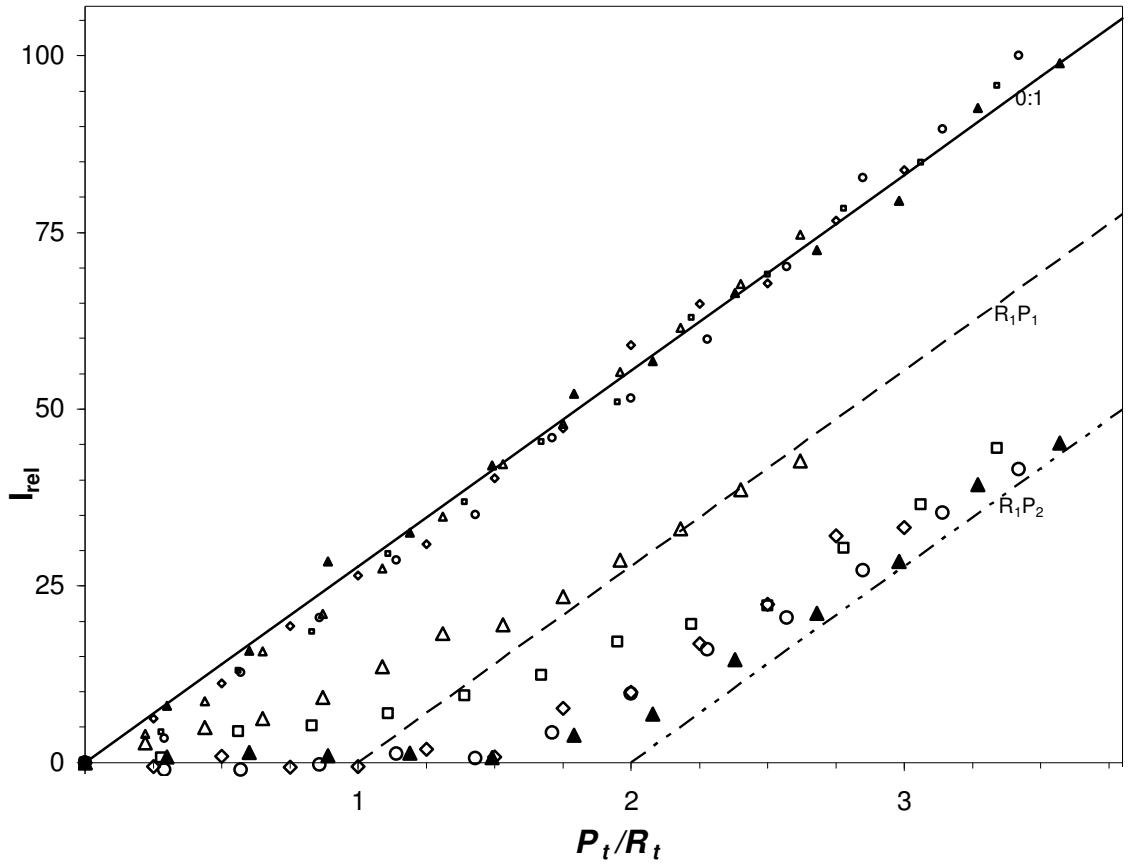

Figure S4.