Supplementary Material

NC competes with Mg^{2+} and Na^{+} for NA binding as an effectively trivalent cation.

Non-specific electrostatic binding between NC and NA plays a large role in NC-facilitated hairpin annealing. This mode of binding assumes that NC is a simple cation with the effective charge z, (*z*-valent), that binds polyanionic NA to screen its charge [1-5]. When several cationic species are present in solution, they compete for NA binding [4-7]. The NC binding isotherm is expected to have a form that is similar to the McGhee and Von Hippel binding isotherm with ligand site exclusion [6; 7]:

$$\Theta_{NC} = \frac{[NC]}{K_d^{NC}} \cdot \left(1 - \Theta_{NC}\right)^z, \qquad \text{(A1)}$$

The K_d^{NC} is defined by either the Mg^{2+} or Na^{+} concentration, depending on which ion is in excess in solution:

$$K_d^{NC} = \frac{[Na]^z}{B} \quad \text{or} \quad K_d^{NC} = \frac{[Mg]^{z/2}}{B} \qquad \text{(A2)}$$

Here, *B* is a factor related to the non-electrostatic component of free energy of NC/NA binding, μ:

$$B = e^{-\mu/RT}, \qquad \text{(A3)}$$

where *R* is the molar gas constant and *T* is temperature in *K*. The effective charge of NC, *z,* can be determined experimentally as the log-log slope of the dissociation constant of NC in Na^{+}:

$$z = \frac{d \log K_d^{NC}}{d \log[Na^+]}, \qquad \text{(A4)}$$

Based on previous NC binding studies, the value for z is 3 ± 0.5 [8-11]. Thus, NC behaves as a trivalent cation, and its dissociation constant is predicted to be $\sim[Na]^3$ or $\sim[Mg]^{3/2}$, i.e., 3 Na^+ or 3/2 Mg^{2+} cations are released from NA upon NC binding.

To estimate Θ_{NC} at any [Mg^{2+}] and [Na^+] using equation (A1), B must be determined. Using a fluorescence polarization binding assay performed in 50 mM NaCl, the K_d for NC binding to a 20-nt ssDNA was determined to be ~80 nM [12]. According to the first equation in (A2), this corresponds to a value of B (~1600). A somewhat higher B=4000 value can be estimated based on other binding measurements [10]. According to equation (A3) $B = 4000$ corresponds to $\mu = -RT \cdot \ln(B) = -(8.3 \pm 0.2)RT = -(5 \pm 0.2)kcal/mol$. The latter is in good agreement with the estimate of -5.2 kcal/mol obtained from direct binding studies by extrapolating measured K_d([Na]) to 1 M salt when all non-electrostatic contributions to binding are expected to vanish[10]. This value is also consistent with the difference in free energy of binding to NA of WT and zinc-less NC [10]. The later observation supports the hypothesis that the non-electrostatic contribution to NC/NA binding comes from stacking of the aromatic residues of NC's zinc fingers with unpaired NA bases [11; 13-16].

HIV-1 NC-induced NA/NA attraction is electrostatic in nature.

The exact nature of NA self-attraction induced by NC is still unclear. It was suggested to be a consequence of attractive interactions between NA-bound NC proteins[17]. However, direct binding data suggest that NC binding to NA is non-cooperative [10; 18], and attractive interactions between the highly cationic NC molecules

have not been observed, either when bound to NA or in solution. An alternative explanation of NC-induced NA aggregation emphasizes the cationic nature of NC and its predominantly electrostatic binding mode to NA. It is well-known that multivalent cations with charge >2 can produce nonspecific NA aggregation [19-23] by a purely electrostatic mechanism [24-30]. Therefore, as an effectively trivalent cation, NC^{3+} is expected to be similar to spermidine^{3+} or cobalt hexamine^{3+} in its NA aggregating properties. These polyvalent cations are known to aggregate single, double or triple stranded nucleic acids [31-35]. The attraction between nucleic acid molecules in this model requires nearly saturated multivalent cation binding [19], and is proportional to the total number of charges, i.e., number of nucleotides in each molecule. Therefore, the attraction between NA molecules is expected to depend on the fraction of nucleic acid saturation with NC, Θ_{NC}, and to be weaker for shorter NA oligomers. The accuracy of our aggregation measurements was insufficient to observe the difference in Θ_{NC} required for aggregation of TAR vs mini-TAR molecules. In both cases the fractional hairpin aggregation, f_a, was close to the fractional NC binding, Θ_{NC}. This suggests that an almost complete NA saturation with NC is required for NA aggregation, consistent with its electrostatic nature.

The fact that even the short 27-nt mini-TAR hairpins are aggregated by saturated NC binding at total hairpin concentration of ~100 nM, but not at lower concentrations, can be used to estimate the attractive energy between NA molecules induced upon NC binding:

$$N \cdot \varepsilon = RT \cdot \ln\left(\frac{C_0}{N \cdot C}\right) \qquad \text{(B1)}$$

Here, N is the number of nt per NA molecule, and ε is the attractive free energy per nt. The right hand side of equation (B1) describes the NA's free energy change due to an entropy loss upon going from the bulk solution at concentration C to an aggregated maximal density state, where the concentration equals the reciprocal NA volume, N/C_0. Here C_0 is the reciprocal volume of a single nucleotide, $C_0 = 1/\pi r^2 b \approx 3M$ (r~1 nm and b~0.17 nm are the ss NA radius and length per nt, respectively). Substituting the numbers typical for our experiment into equation (B1), i.e., C~100 nM and N=27, an estimate of the attractive free energy per nt can be obtained: $\varepsilon \approx 0.4$ RT ≈ 0.3 kcal/mol. This estimate is significantly higher than previously estimated values of 0.02 RT, 0.08 RT and 0.07RT for dsDNA aggregation by sperimdine^{3+}, cobalt hexamine^{3+} and spermine^{4+}, respectively [25]. Nevertheless, the estimated ε value for NC is still within the range that is reasonable for electrostatic interactions between trivalent cations and NA phosphate charges [24; 25; 27]. The stronger NC-induced NA attraction compared to that induced by polyamines with approximately the same effective charge (z~3) may be a result of a more compact charge distribution on NC in its N-terminal domain. It could also be a result of NC's stronger binding to NA due to non-electrostatic interactions via the zinc-finger domains.

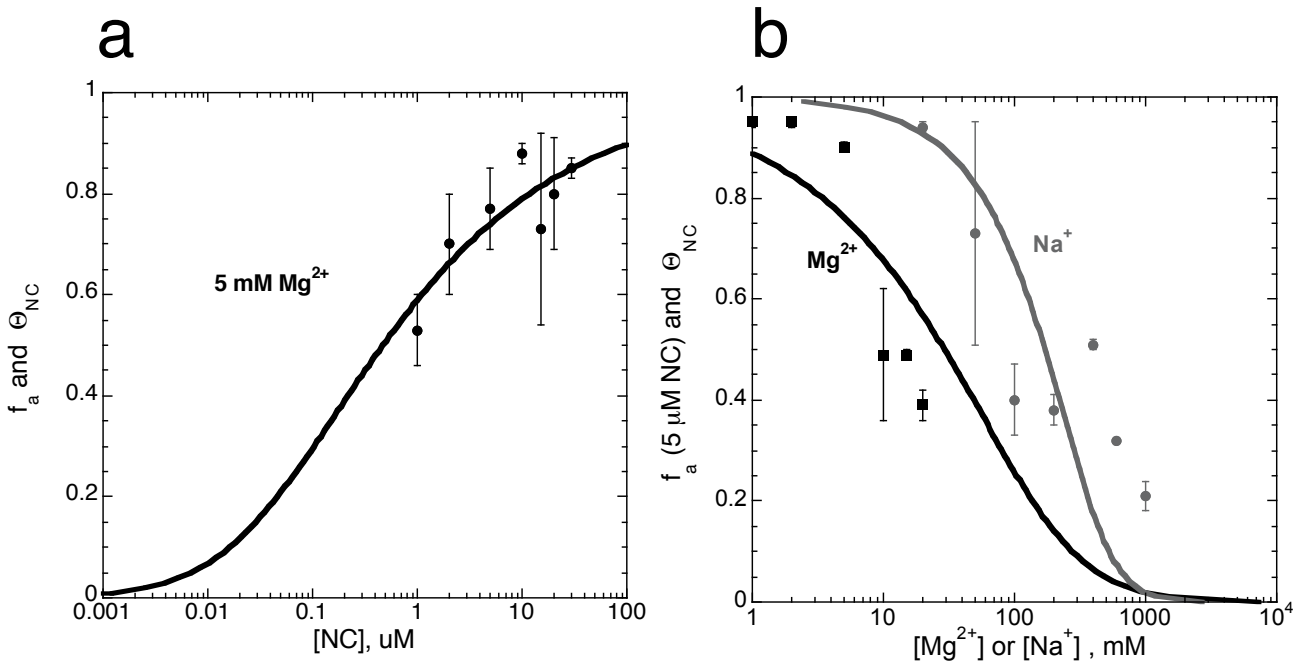

Figure S1 Fraction of RNA aggregated (f_a) measured by sedimentation (data points), and fraction of NC bound (Θ_{NC}) calculated according to eqs. (A1-A3) (solid lines). Experiments were conducted in the presence of 5 mM Mg^{2+} as a function of various concentrations of NC (panel a); and in the presence of 5 μM NC as a function various concentrations of Na^+ or Mg^{2+} (panel b). Circles and squares correspond to mini-TAR and full-length TAR RNA, respectively.

References

1. Cantor, C. R. & Schimmel, P. R. (1980). *Biophysical Chemistry. Part III. The Behavior of Biological Macromolecules*, W. H. Freeman & Co., San Francisco.
2. Bloomfield, V. A., Crothers, D. M. and Tinoco, I. (2000). *Nucleic Acids Structure, Properties, and Function*, University Science Books, Sausalito, CA.
3. Frank-Kamenetskii, F. (1971). Simplification of the empirical relationship between melting temperature of DNA, its GC content and concentration of sodium ions in solution. *Biopolymers* **10**, 2623-4.
4. Gueron, M. & Weisbuch, G. (1981). Polyelectrolyte theory of charged-ligand binding to nucleic acids. *Biochimie* **63**, 821-5.
5. Manning, G. S. (1978). The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Q Rev Biophys* **11**, 179-246.
6. Rouzina, I. & Bloomfield, V. A. (1997). Competitive electrostatic binding of charged ligands to polyelectrolytes: practical approach using the non-linear Poisson-Boltzmann equation. *Biophys Chem* **64**, 139-55.
7. Rouzina, I., and Bloomfield, V. A. (1996). Competitive electrostatic binding of charged ligands to polyelectrolytes: Planar and cylindrical geometries. *J Phys Chem* **100**, 4292-4304.
8. Paoletti, A. C., Shubsda, M. F., Hudson, B. S. & Borer, P. N. (2002). Affinities of the nucleocapsid protein for variants of SL3 RNA in HIV-1. *Biochemistry* **41**, 15423-8.

9. Urbaneja, M. A., Wu, M., Casas-Finet, J. R. & Karpel, R. L. (2002). HIV-1 nucleocapsid protein as a nucleic acid chaperone: spectroscopic study of its helix-destabilizing properties, structural binding specificity, and annealing activity. *J Mol Biol* **318**, 749-64.

10. Vuilleumier, C., Bombarda, E., Morellet, N., Gerard, D., Roques, B. P. & Mély, Y. (1999). Nucleic acid sequence discrimination by the HIV-1 nucleocapsid protein NCp7: a fluorescence study. *Biochemistry* **38**, 16816-25.

11. Yuan, Y., Kerwood, D. J., Paoletti, A. C., Shubsda, M. F. & Borer, P. N. (2003). Stem of SL1 RNA in HIV-1: structure and nucleocapsid protein binding for a 1 x 3 internal loop. *Biochemistry* **42**, 5259-69.

12. Stewart-Maynard, K. M., Cruceanu, M., Wang, F., Vo, M. N., Gorelick, R. J., Williams, M. C., Rouzina, I. & Musier-Forsyth, K. (2008). Retroviral NC proteins display non-equivalent levels of nucleic acid chaperone activity. *J Virol,, in press.*

13. De Guzman, R. N., Wu, Z. R., Stalling, C. C., Pappalardo, L., Borer, P. N. & Summers, M. F. (1998). Structure of the HIV-1 nucleocapsid protein bound to the SL3 Ψ-RNA recognition element. *Science* **279**, 384-8.

14. Amarasinghe, G. K., De Guzman, R. N., Turner, R. B. & Summers, M. F. (2000). NMR structure of stem-loop SL2 of the HIV-1 psi RNA packaging signal reveals a novel A-U-A base-triple platform. *J Mol Biol* **299**, 145-56.

15. Amarasinghe, G. K., Zhou, J., Miskimon, M., Chancellor, K. J., McDonald, J. A., Matthews, A. G., Miller, R. R., Rouse, M. D. & Summers, M. F. (2001). Stem-loop SL4 of the HIV-1 psi RNA packaging signal exhibits weak affinity for the

nucleocapsid protein. structural studies and implications for genome recognition. *J Mol Biol* **314**, 961-70.

16. Beltz, H., Clauss, C., Piemont, E., Ficheux, D., Gorelick, R. J., Roques, B., Gabus, C., Darlix, J. L., de Rocquigny, H. & Mély, Y. (2005). Structural determinants of HIV-1 nucleocapsid protein for cTAR DNA binding and destabilization, and correlation with inhibition of self-primed DNA synthesis. *J Mol Biol* **348**, 1113-26.

17. Darlix, J. L., Lapadat-Tapolsky, M., de Rocquigny, H. & Roques, B. P. (1995). First glimpses at structure-function relationships of the nucleocapsid protein of retroviruses. *J Mol Biol* **254**, 523-37.

18. Mély, Y., de Rocquigny, H., Sorinas-Jimeno, M., Keith, G., Roques, B. P., Marquet, R. & Gerard, D. (1995). Binding of the HIV-1 nucleocapsid protein to the primer tRNA(3Lys), in vitro, is essentially not specific. *J Biol Chem* **270**, 1650-6.

19. Bloomfield, V. A. (1997). DNA condensation by multivalent cations. *Biopolymers* **44**, 269-82.

20. Heilman-Miller, S. L., Pan, J., Thirumalai, D. & Woodson, S. A. (2001). Role of counterion condensation in folding of the Tetrahymena ribozyme. II. Counterion-dependence of folding kinetics. *J Mol Biol* **309**, 57-68.

21. Koculi, E., Lee, N. K., Thirumalai, D. & Woodson, S. A. (2004). Folding of the Tetrahymena ribozyme by polyamines: importance of counterion valence and size. *J Mol Biol* **341**, 27-36.

22. Koculi, E., Thirumalai, D. & Woodson, S. A. (2006). Counterion charge density determines the position and plasticity of RNA folding transition states. *J Mol Biol* **359**, 446-54.

23. Koculi, E., Hyeon, C., Thirumalai, D. & Woodson, S. A. (2007). Charge density of divalent metal cations determines RNA stability. *J Am Chem Soc* **129**, 2676-82.

24. Rouzina, I., and Bloomfield, V. A. (1996). Macroion attraction due to electrostatic correlation between screening counterions. 1. Mobile surface-adsorbed ions and diffuse ion cloud. *J Phys Chem* **100**, 9977-9989.

25. Nguyen, T. T., Rouzina, I., and Shklovskii, B. I. (2000). Reentrant condensation of DNA induced by multivalent counterions. *J Chem Phys* **112**, 2562-2568.

26. Nguyen, T. T. & Shklovskii, B. I. (2002). Model of inversion of DNA charge by a positive polymer: fractionalization of the polymer charge. *Phys Rev Lett* **89**, 018101.

27. Zhang, R. & Shklovskii, B. I. (2004). Phase diagram of aggregation of oppositely charged colloids in salty water. *Phys Rev E Stat Nonlin Soft Matter Phys* **69**, 021909.

28. Lau, A. W., Pincus, P., Levine, D. & Fertig, H. A. (2001). Electrostatic attraction of coupled Wigner crystals: finite temperature effects. *Phys Rev E Stat Nonlin Soft Matter Phys* **63**, 051604.

29. Lobaskin, V., Lyubartsev, A. & Linse, P. (2001). Effective macroion-macroion potentials in asymmetric electrolytes. *Phys Rev E Stat Nonlin Soft Matter Phys* **63**, 020401.

30. Linse, P., and Lobaskin, V. (2000). Electrostatic attraction and phase separation in solutions of like-charged colloidal particles. *J Chem Phys* **112**, 3917-3927.

31. Arscott, P. G., Ma, C., Wenner, J. R. & Bloomfield, V. A. (1995). DNA condensation by cobalt hexaammine (III) in alcohol-water mixtures: dielectric constant and other solvent effects. *Biopolymers* **36**, 345-64.

32. Kabanov, A. V. & Kabanov, V. A. (1995). DNA complexes with polycations for the delivery of genetic material into cells. *Bioconjug Chem* **6**, 7-20.

33. Pelta, J., Jr., Durand, D., Doucet, J. & Livolant, F. (1996). DNA mesophases induced by spermidine: structural properties and biological implications. *Biophys J* **71**, 48-63.

34. Pelta, J., Livolant, F. & Sikorav, J. L. (1996). DNA aggregation induced by polyamines and cobalthexamine. *J Biol Chem* **271**, 5656-62.

35. Raspaud, E., Olvera de la Cruz, M., Sikorav, J. L. & Livolant, F. (1998). Precipitation of DNA by polyamines: a polyelectrolyte behavior. *Biophys J* **74**, 381-93.