Supporting Information

The essential role of stacking adenines in a two-base-pair RNA kissing complex

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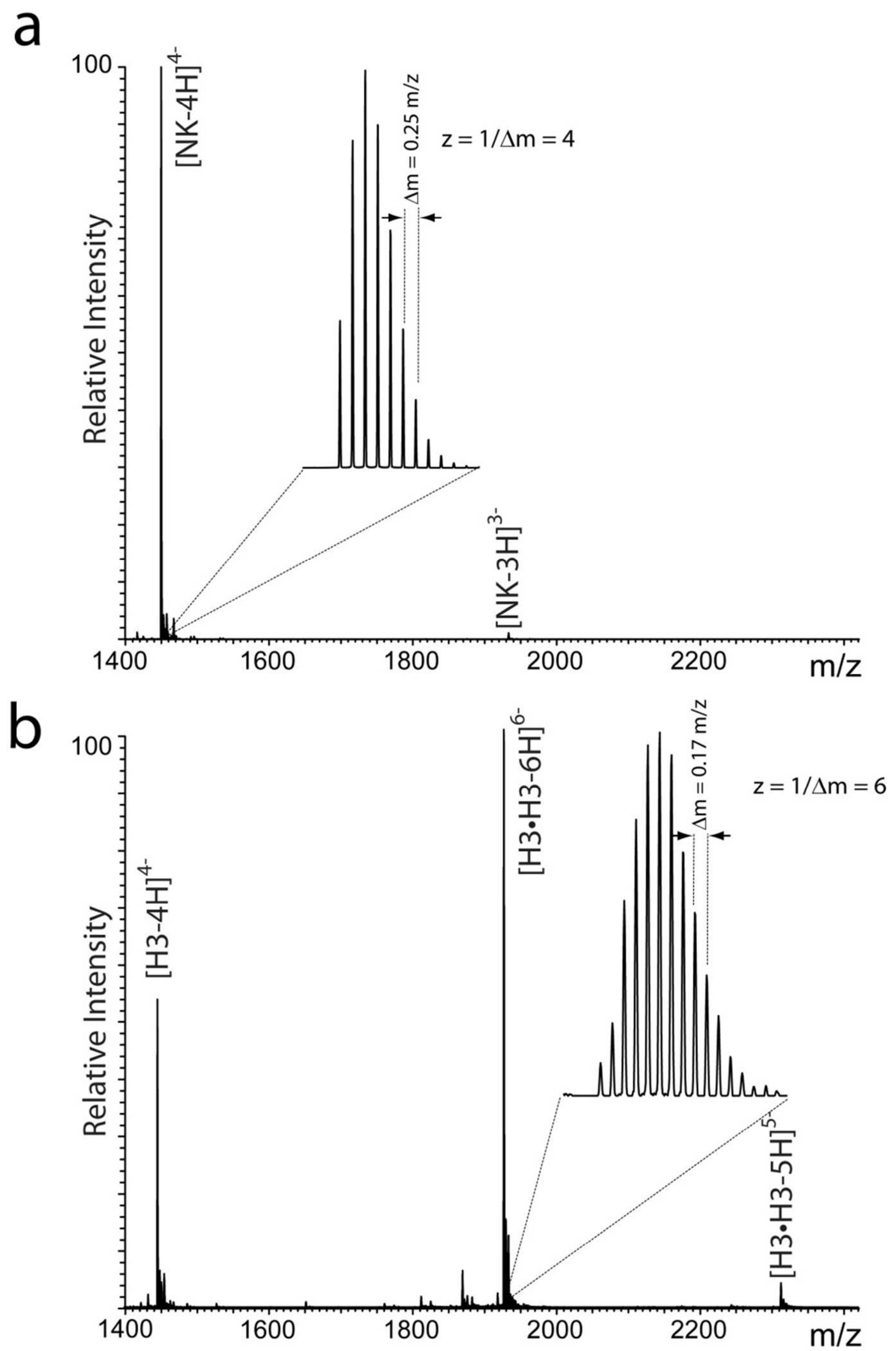

Figure S1. ESI-MS spectra obtained from a) NK and b) H3 RNA (see Experimental Methods for conditions). Charge states were determined from the spacing of the respective isotopic distributions (insets), which enabled also the unambiguous discrimination between monomers and dimers with twice the charge. Only monomeric form was detected in the NK sample with a 5802.28 Da experimental mass (5802.55 Da calculated from sequence). H3 RNA was detected in both monomeric (5785.32 Da exp., 5785.57 Da calc.) and dimeric form (11571.66 Da exp., 11571.14 Da calc.).

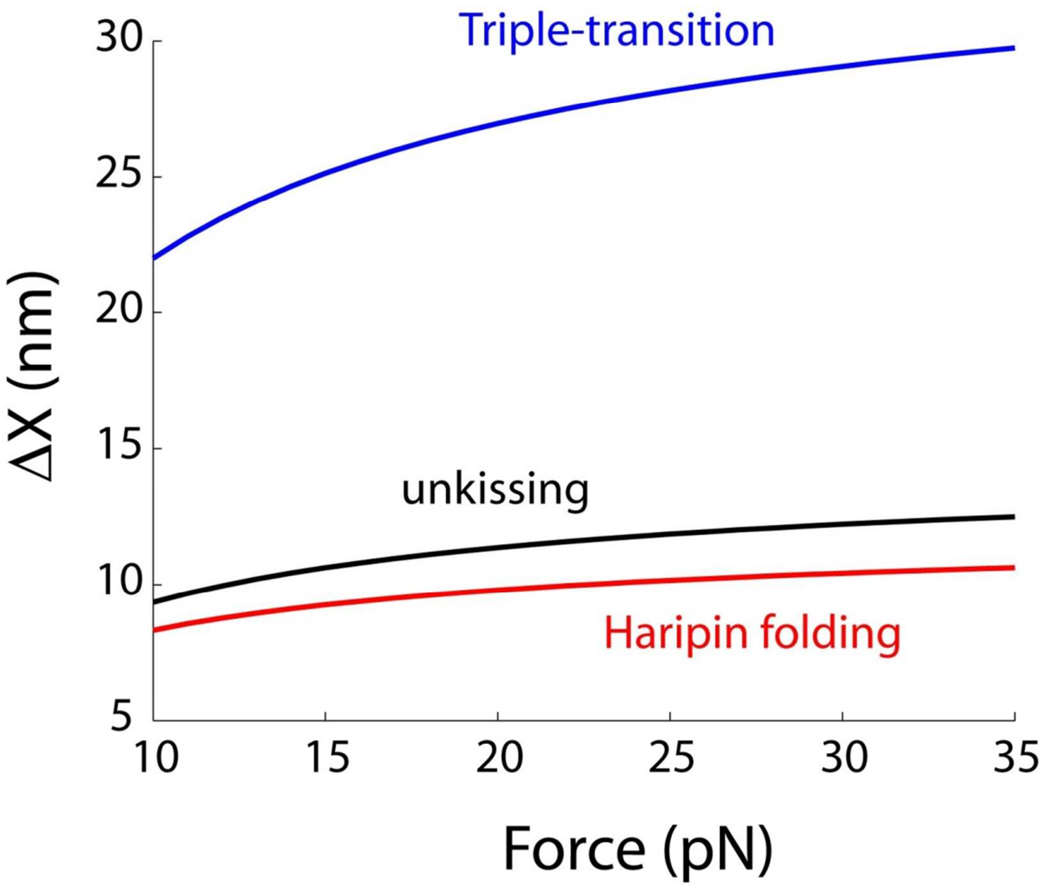

Figure S2. Predicted ΔX for hairpin (un)folding, unkissing, and the tripele-transition as a function of force. The prediction was based on a method described previously.[1, 2] Briefly, we estimated extensions of each of the structures along the folding pathway, i.e., the kissing complex, 2-hp, 1-hp, and single-stranded. Then we computed ΔX for each structural transition. Specifically, we used a worm-like-chain model[3] with the persistence length of 1 nm for single-stranded RNA, 2 nm for the width of a helix, and 6 nm for the extension of the kissing complex. The estimations worked reasonably well. For instance, ΔX of the hairpins was observed at ~8 nm at 18 pN in 200 mM NaCl, as compared to 9 nm by calculation.

1. Li PT, Tinoco I, Jr. Mechanical unfolding of two DIS RNA kissing complexes from HIV-1. J Mol Biol 2009; 386:1343-56.
2. Li PTX, Bustamante C, Tinoco I, Jr. Unusual Mechanical Stability of A Minimal RNA Kissing Complex. Proc Natl Acad Sci U S A 2006; 103:15847-52.
3. Bustamante C, Marko JF, Siggia ED, Smith SB. Entropic elasticity of lambda-phage DNA. Science 1994; 265:1599-600.

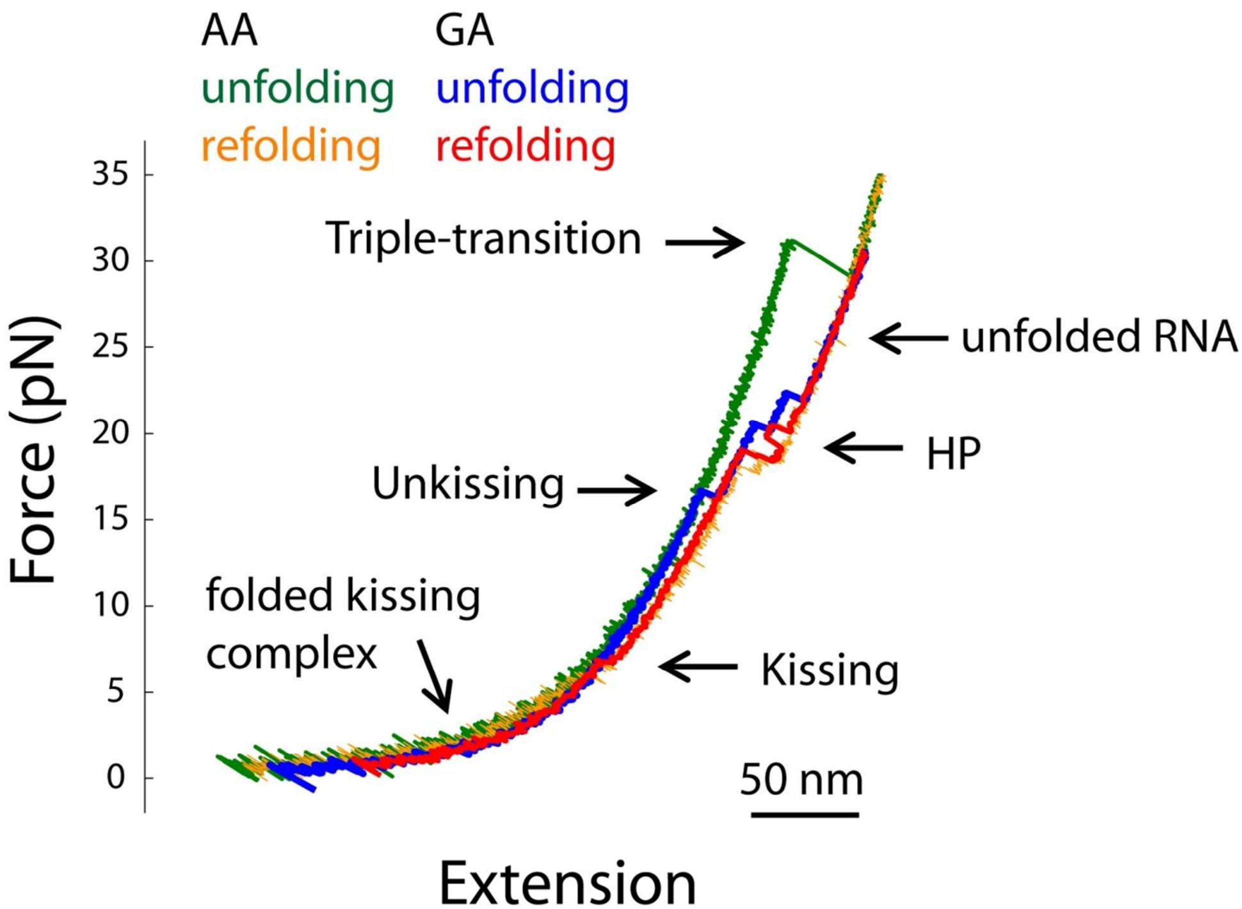

Figure S3. Superimposed AA and GA force-extension curves. On force relaxation, the folded kissing complex (at low force) and fully unfolded RNA (at high forces) of the two RNAs showed similar force-extension relations. Furthermore, hairpin refolding and the kissing formation in the two RNAs showed similar transitions. When force was increased, the GA showed three unfolding transitions. Before the first unfolding, the force-extension curve of the GA followed closely to that of the AA. The unfolding transitions of the hairpins in the GA were comparable to the refolding signals. These observations suggested that the first unfolding transition in the GA molecule was the unkissing.

1. Li PT, Tinoco I, Jr. Mechanical unfolding of two DIS RNA kissing complexes from HIV-1. J Mol Biol 2009; 386:1343-56.
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