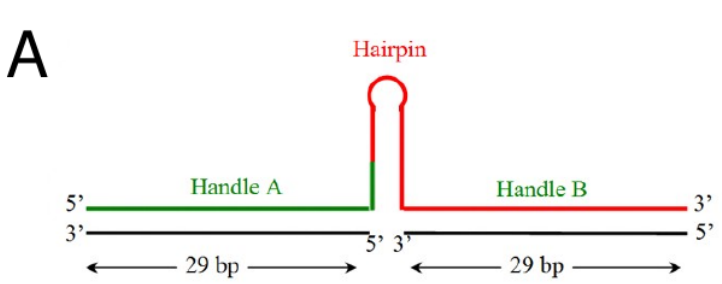
Supplemental Material to "Counterpropagating dual–trap optical tweezers based on linear momentum conservation".

M. Ribezzi–Crivellari,[1] J. M. Huguet,[1] and F. Ritort[1,2]

[1] *Departament de Fisica Fonamental, Universitat de Barcelona*

[2] *Ciber-BBN de Bioingeneria, Biomateriales y Nanomedicina*



B

Name	Sequence
SPLINT	5' GCGCTGGCACTGTGTTTCCACCACTAACT 3'
HandleA	5'Biotin-AGTTAGTGGTGGAAACACAGTGCCAG CGCGCGAGCCATAAT3'
CD4-5 HandleB	5'Pho-TCATCTGGAAAACAGATGAGATTATGGC TCGCAGTTAGTGGTGGAAACACAGTGCCAGCGC 3'

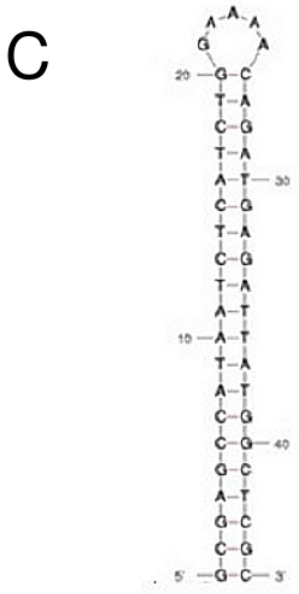


FIG. 1. A) The hairpin was synthesized using three different oligos, (Handle A, Handle B and Splint) [1] B) Sequence of the three oligos used in the synthesis C) Schematic Representation of the stem and loop of folded hairpin, molecular handles are not shown.

I. SAMPLE PREPARATION

The DNA haripins used in the experiments reported in the Main Text have a 20 bp stem and 5 or 8 bases loop. This hairpin had the open ends of the stem linked to 29 bp double stranded DNA handles which act as spacers. The free ends of the DNA handles were marked with digoxigenin on one end and with biotin at the other end. The hairpin sequence is schematically represented in Fig. S2. Syntesis and characterization of similar molecules is described in [1].

For the stiffness measurements he 24kbp ds–DNA was obtained by digestion from the genome of phage λ. Also in this case the ends of the molecule were marked with biotin and digoxigenin In order to manipulate the molecules these had the two extremes chemically linked to 4 μm Silica beads. Beads (Kisker Biotech) were coated with either antidigoxigenin or streptavidin. The hydrodynamic measurements were performed on the hairpins, on the 24 kbp ds-DNA and on two other thethers of 3kbp and 1.2 kbp respectively. These tethers were

obtained by digestion and PCR amplification of the phage λ genome. All experiments were performed in a microfluidics chamber formed by two coverslips interspaced with parafilm. The chamber has three channels: a central one where experiments are carried out and two (upper and lower) channels that are connected to the central one by two dispenser tubes. Anti-dig coated beads were first incubated with the molecule of interest and then introduced in the microfluidics chamber through one of the dispenser tubes. Once the anti-dig coated bead was trapped a streptavidin coated bead was introduced through a second dispenser tube and trapped in the second trap. The connection was then formed directly inside the microfluidics chamber. All experiments on DNA tethers were performed in PBS buffer solution at pH 7.4, 1M NaCl, at 25^o C. This buffer solution was found to greatly reduce the nonspecific adsorption of DNA on silica, allowing the use of commercial beads from Kisker Biotech without any specific preparation or coating. We dissolved $1mg/\mu l$ Bovine Serum Albumine in the buffer in order to reduce silica-silica interactions.

II. RECONSTRUCTION OF A COARSE FREE ENERGY LANDSCAPE

The mechanical folding and unfolding of nucleic acid hairpins is commonly described in terms of a reaction coordinate and of the corresponding free energy landscape. When subject to force, the end-to-end distance of the molecule along the force axis is an adequate reaction coordinate for the folding-unfolding reaction pathway. For a given applied force f, a single kinetic pathway for the unfolding and folding reactions is usually considered, characterized by a single transition state (TS). The TS is the highest free–energy state encountered along the reaction coordinate and determines the kinetics of the folding-unfolding reaction. The model involves four parameters: the free energy of folding at zero force, $\Delta G = G_F - G_U$, the height of the kinetic barrier B_0, defined as the free energy difference between the transition state and the folded (F) state extrapolated to zero force, and the distances x_F and x_U along the reaction coordinate that separates the transition state from states F and U respectively; the total distance along the reaction coordinate being $x_m = x_F + x_U$. Under an applied force the free energy landscape is tilted along the reaction coordinate and the free–energy difference ΔG and the barrier B_0 change accordingly. To a first approximation, ΔG and B depend linearly on the force whereas x_F and x_U are taken force–independent. Hence the

reaction rates are given by

$$k_{F\to U} = k_0 e^{-\beta(B-G_F-x_{FU}f)} = k_m e^{\beta x_{\mathrm{FU}f}} \tag{1}$$

$$k_{U\to F} = k_0 e^{-\beta(B-G_U+x_{UF}f)} = k_m e^{\beta(\Delta G_0 - x_{\mathrm{UF}}f)}, \tag{2}$$

and $k_m = k_0 e^{-\beta B_0}$ is an effective attempt rate. The free–energy difference between state U and F under the given force f is given by,

$$\Delta G(f) = -k_B T \log\left(\frac{k_{F\to U}}{k_{U\to F}}\right) = \Delta G_0 - x_m f. \tag{3}$$

with $\Delta G_{FU}(f) = G_F(f) - G_U(f)$. The four parameters describing the free–energy landscape can therefore be reconstructed from the kinetic rates measured at different forces.

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[1] N. Forns, S. de Lorenzo, M. Manosas, K. Hayashi, J.M. Huguet and F. Ritort, Biophysical Journal **100**, 1765 (2011)