FORCE KINETICS OF DESIGNED DNA HAIRPINS WITH INTERMEDIATE AND MISFOLDED STATES INVESTIGATED USING OPTICAL TWEEZERS

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Pulling experiments are being used to investigate many molecular processes by exerting mechanical force on individual molecules like DNA, RNA and proteins. Force and extension measurements make possible to determine the folding free energies and kinetic parameters of biomolecules and the search for intermediates and misfolded states as well. Here we investigate folding and unfolding force kinetics of DNA hairpins of specifically designed sequences using optical tweezers (Huguet *et al*., 2008).

We have designed specific DNA hairpins with different free energy landscapes in order to study their force folding kinetics in the presence of intermediate states on-pathway and off-pathway. We have synthesized three kinds of hairpins: one hairpin folds and unfolds in a cooperative two-states manner; another hairpin has an intermediate state on-pathway; finally, the third hairpin has a misfolded state off-pathway.

We have carried out hopping experiments under two conditions: constant force mode experiments and passive mode experiments (Wen *et al*., 2007; Manosas *et al*., 2007). In these experiments it is possible to determine the reaction rates at different forces by measuring the residence time of the molecule in each state. In this way we are able to precisely determine the kinetic parameters of the free energy landscape, the force folding kinetics and the free energies of formation of the different structures.

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