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Single-molecule FRET studies of RNA folding and catalysis

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Single-molecule FRET has been applied to the folding of branched DNA and RNA structures, and their dynamics. We find that FRET efficiencies from single junctions encapsulated within phospholipid vesicles are more reliable than those from ensemble measurements, and a valuable source of structural data. We have shown that both cyanine fluorophores are substantially stacked upon the end of double-stranded DNA or RNA when attached at the 5'-terminus. This leads to a significant orientation dependence of FRET efficiency that is incompletely averaged by lateral motion of the fluorophores. Using a series of DNA and RNA duplexes we have shown that the orientation dependence leads to a pronounced modulation of efficiency as a function of helix length; these data unequivocally establish that Förster transfer obeys the orientation dependence as expected for a dipole-dipole interaction Ignoring this effect can lead to significant errors in distance determination. However, a full understanding of the orientation dependence could greatly extend the use of FRET measurements to provide both accurate distance and angular information.