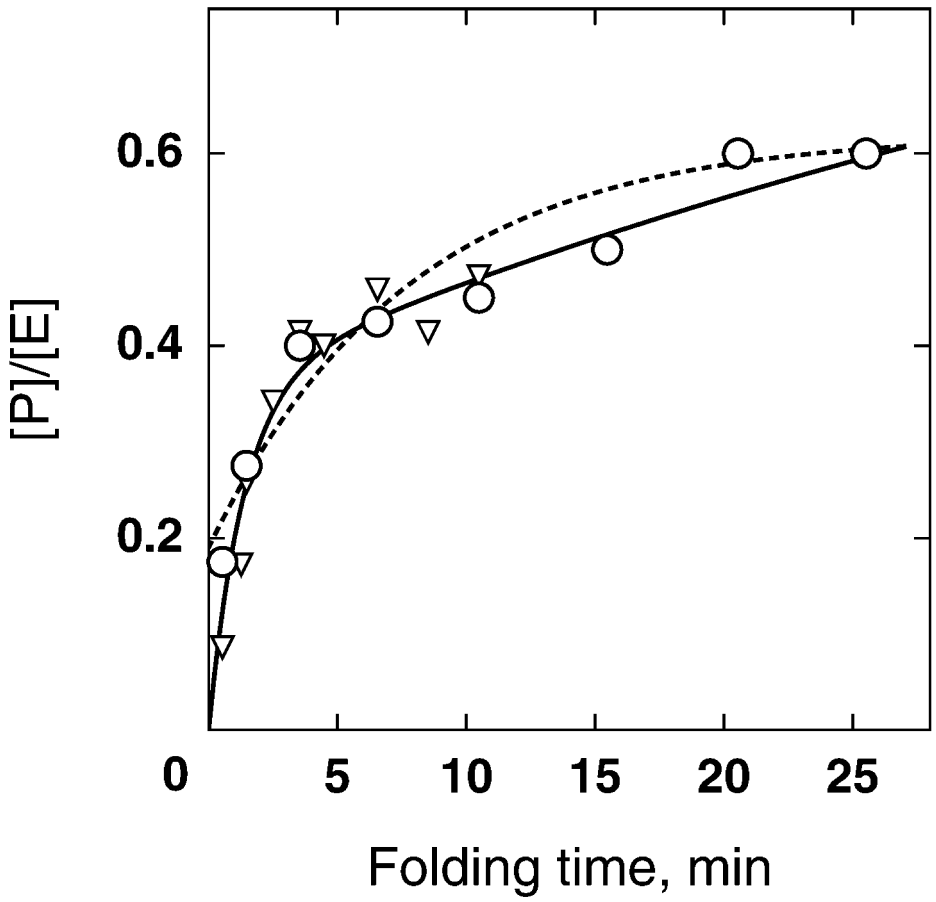
0.6
0.4
0.2
[P]/[E]
0
5
10
15
20
25
Folding time, min

Supplementary Figure. Previous ribozyme folding experiments give biphasic folding to the native state. Data are from refs. 9,10 (), obtained by monitoring the onset of enzymatic cleavage activity of the ribozyme (E), in which the oligonucleotide substrate is cleaved to the shorter product (P). Data were previously interpreted in the context of a model with a single folding population giving a single rate constant (broken line). However, this fit gave a positive y-intercept of 0.2 instead of passing through zero product formed at zero time, suggesting that 20% of the population folded with a larger rate constant. The fit also gave a final folded fraction of ~0.6, suggesting that a fraction of the population folded with a smaller rate constant than obtained from the fit by a single exponential. The fit also gave systematic deviation from the data. Fitting these data with the sum of two exponentials (solid line) gives a reasonable fit with the initial and ending values of the fraction of native ribozyme fixed at 0 and 1, respectively, as expected,[11] and reduces the systematic deviation. The rate constants obtained are 0.7 min^{-1} and 0.02 min^{-1}. The larger rate constant is the same within error as observed by Zarrinkar & Williamson[1] using the same assay, and the data from the earlier work () are essentially superimposable with those of Rook *et al*.[9,10] The presence of two folding phases is consistent with data presented herein and with the model of Figure 4, which predicts that a fraction of the population folds to the native state from I_{trap} with a rate constant of 1.5 min^{-1} while the rest of the population slowly re-folds from the misfolded form to the native form (10^{-3} min^{-1}). The difference in magnitude of the smaller rate constant (0.02

min^{-1} here vs 10^{-3} min^{-1} in Fig. 2c) may be due to small differences in cation concentrations (see text). To prepare this figure, data from Rook *et al*.[9] and Zarrinkar & Williamson,[1] reported in concentration of oligonucleotide product, were normalized to the ribozyme concentration. The folding times of all data were adjusted by adding the time allowed for the cleavage reaction, 30 s, to the reported folding times. This adjustment was necessary because these experiments were performed by first allowing the ribozyme to fold for various amounts of time and then adding S and incubating for an additional 30 s. Because additional ribozyme is expected to fold and rapidly cleave S during this 30 s,[11,22] it is appropriate to include this 30 s in the reported folding time.