Electronic Supplementary Material (ESI) for Nanoscale.

Single Molecule Force Spectroscopy Reveals That Two-coordinate Ferric Site Is Critical for The Folding of Holo-rubredoxin

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Supplementary Information

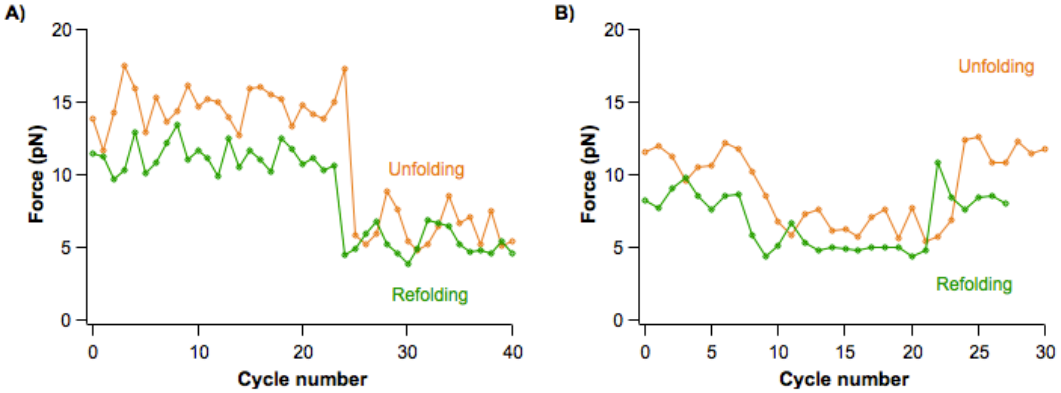

Figure S1. Unfolding (orange) and refolding (green) forces of two apo-RD molecules in consecutive unfolding-refolding cycles. The synchronous changes of unfolding and refolding forces indicate the switch of apo-RD between apo-RD_H and apo-RD_L.

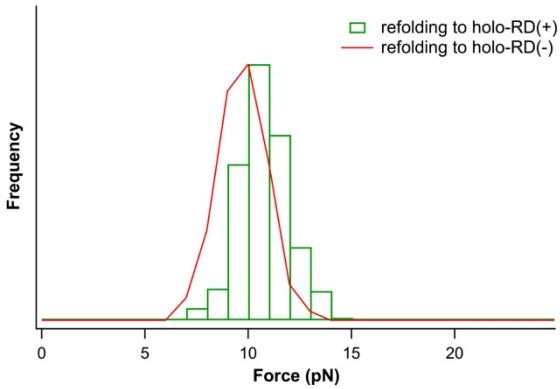

Figure S2. Refolding force distribution of unfolded holo-RD_H leading to holo-RD(+) versus those leading to holo-RD(-). Both histograms have the same bin size (1 pN). For clarity, only the refolding to holo-RD(+) data is displayed as a bar chart.

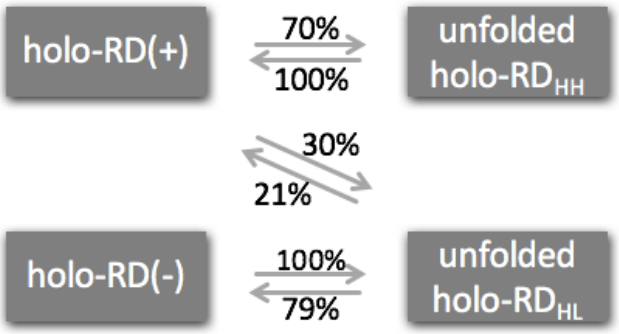

Figure S3. 4-state Markov chain model for unfolding and refolding of holo-RD. The transition probability between states is calculated from experimental data and indicated in the schematics.

Table S1. Calculated and experimental occupancy of each species in the unfolding-refolding process of holo-RD.

	holo-RD(+)	holo-RD(-)	unfolded holo-RD$_{HH}$	unfolded holo-RD$_{HL}$
Calculated occupancy	0.47	0.53	0.33	0.67
Experimental occupancy	0.39	0.61	0.30	0.70