

Supporting Information

Mechanistic insights into poly(C)-binding protein hnRNP K resolving i-motif

DNA secondary structures

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Tables S1-S3

Figures S1-S7

Table S1. Sequences of substrates used in the experiments.

Name	For figures	Sequences (5'-3') of substrates for CD
Py25	Figure 1	TCCCCACCCTCCCCACCCTCCCC
Py25(1245)	Figures 1 and 2	TCCCCACCCTTTTTACCCTCCCC
Py25(1234)	Figures 1 and 2	TCCCCACCCTCCCCACCCTTTTT
Sequences (5'-3') of substrates for FRET		
Py25'	Figures 3, 4, 5, 9, S2, S3, S5 and S7	T TCCCCACCCTCCCCACCCTCCCCATGAGGACACGTGCATTCC Biotin-GGAATGCACGTG T CCTC
Py25(1245)'	Figures 3, S2, S4 and S5	T TCCCCACCCTTTTTACCCTCCCCATGAGGACACGTGCATTCC Biotin-GGAATGCACGTG T CCTC
Py25(1234)'	Figures 3 and S2	T TCCCCACCCTCCCCACCCTTTTTATGAGGACACGTGCATTCC Biotin-GGAATGCACGTG T CCTC
Py25(1235)'	Figure S2	T TCCCCACCCTCCCCATTTTCCCCATGAGGACACGTGCATTCC Biotin-GGAATGCACGTG T CCTC
Py25(1345)'	Figure S2	T TCCCCATTTTCCCCACCCTCCCCATGAGGACACGTGCATTCC Biotin-GGAATGCACGTG T CCTC
Py25(2345)'	Figure S2	T TTTTTACCCTCCCCACCCTCCCCATGAGGACACGTGCATTCC Biotin-GGAATGCACGTG T CCTC
Py25(245)'	Figures 6 and S6	T TTTTTACCCTTTTTACCCTCCCCATGAGGACACGTGCATTCC Biotin-GGAATGCACGTG T CCTC
Py25(45)'	Figures 7 and S6	T TTTTTATTTTTTTTACCCTCCCCATGAGGACACGTGCATTCC Biotin-GGAATGCACGTG T CCTC
Py25(24)'	Figures 7 and S6	T TTTTTACCCTTTTTACCCTTTTTATGAGGACACGTGCATTCC Biotin-GGAATGCACGTG T CCTC

Color: **Green, Cy3**; **Red, Cy5**. **Bold: C** sequences. Underline: dsDNA forming sequences.

Table S2. Kinetic parameters of hnRNP K resolving the Py25' i-motif at different protein concentrations using bulk fluorescence spectra obtained from the exponential fittings of the data shown in Figure 4C.

Concentration	Unwinding amplitude of Cy3 (a.u.)	Rate constant (min ⁻¹)	Unwinding rate (a.u. min ⁻¹)
4 nM	1451.83 ± 270.29	0.15 ± 0.06	234.63 ± 126.82
20 nM	2128.24 ± 183.29	0.44 ± 0.10	947.84 ± 287.51
40 nM	2297.63 ± 319.21	0.46 ± 0.16	1115.41 ± 524.77
200 nM	10134.70 ± 395.94	0.36 ± 0.04	3644.02 ± 525.37
400 nM	10781.06 ± 618.93	0.51 ± 0.07	5605.80 ± 1113.23
2 μM	16311.28 ± 359.88	0.95 ± 0.05	15547.74 ± 1162.93
4 μM	16337.09 ± 1007.81	1.45 ± 0.25	23869.24 ± 5579.11

Table S3. Kinetic parameters of KH1-3 and hnRNP K resolving the Py25' i-motif at 1 μM protein, as obtained from exponential fittings of the data in Figure 9D.

Protein	Unfolding percent	Rate constant (min ⁻¹)	Unwinding rate (min ⁻¹)
KH1	11.22 ± 1.32	1.93 ± 0.55	22.34 ± 8.74
KH2	53.11 ± 3.27	0.84 ± 0.12	44.88 ± 9.27
KH3	48.46 ± 3.13	0.80 ± 0.12	39.18 ± 8.53
hnRNP K	80.48 ± 6.68	1.25 ± 0.24	101.94 ± 27.57

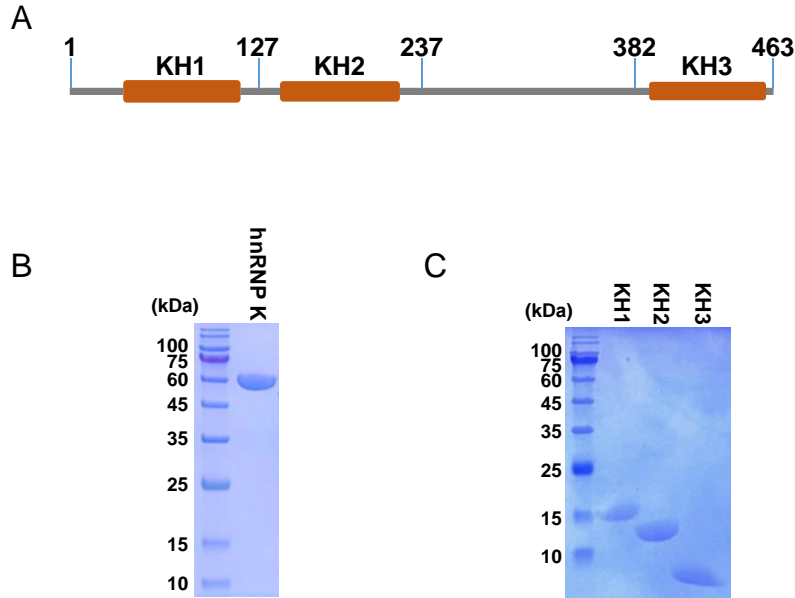


Figure S1. Domain architecture and purification. (A) Diagram of hnRNP K. (B, C) Analysis of the purified hnRNP K (B) and KH1-3 (C) by SDS-PAGE.

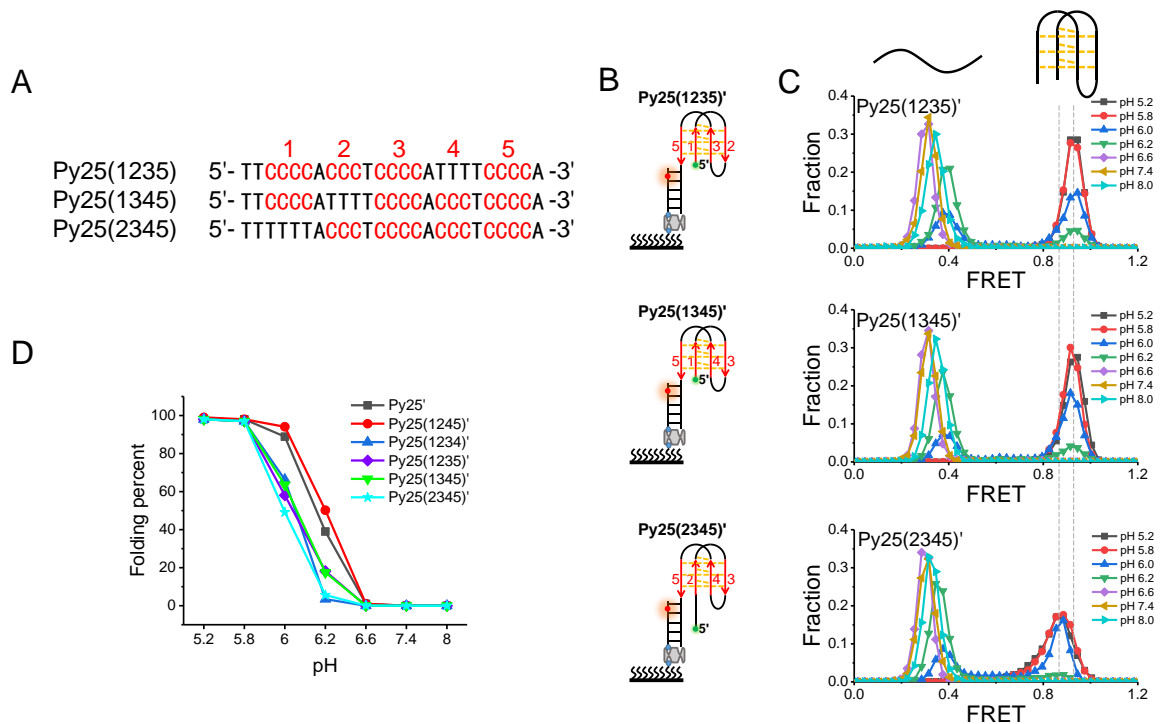


Figure S2. Folding of Py25(1235)', Py25(1345)', and Py25(2345)' at different pH levels from 5.2 to 8.0 using smFRET. (A) The sequences of Py25(1235)', Py25(1345)', and Py25(2345)'. (B, C) Schematic diagram and the FRET distributions at different pH of Py25(1235)', Py25(1345)', and Py25(2345)'. FRET values below 0.4 indicate ssDNA, and FRET values exceeding 0.8 indicate unfolded i-motif DNA (Figure 3). (D) Folding percentages of Py25', Py25(1245)', Py25(1234)', Py25(1235)', Py25(1345)', and Py25(2345)' at different pH values from 5.2 to 8.0, as derived from Figure 3 and (C).

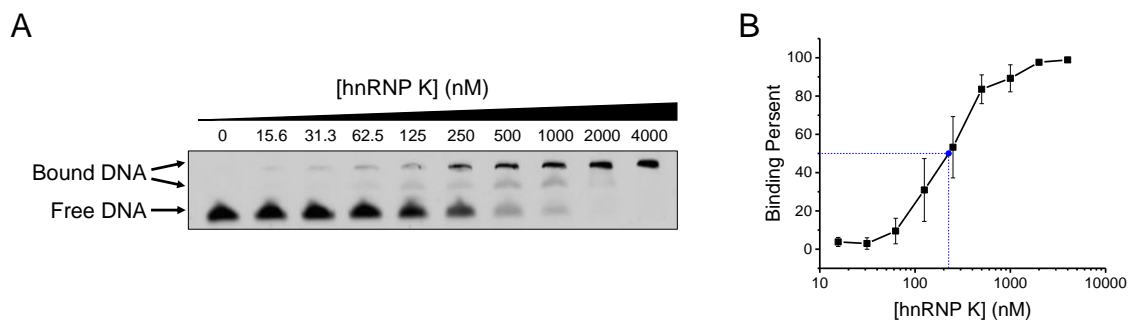


Figure S3. The binding of hnRNP K with Py25'. **(A)** Different concentrations of hnRNP K were mixed with 80 nM Py25', and PAGE bandshift assays determined the binding. **(B)** Quantitative analyses of hnRNP K binding Py25' by ImageJ (n = 3). The apparent DNA binding affinity value was approximately 220 nM.

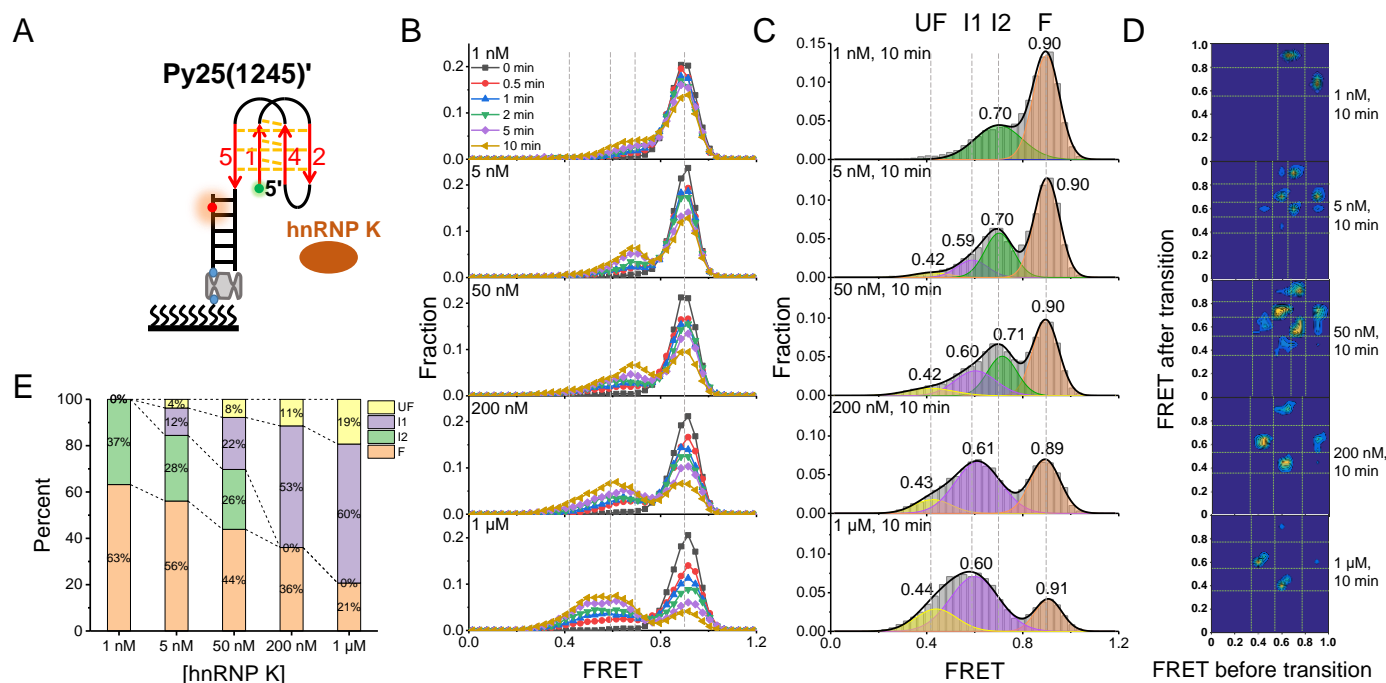


Figure S4. hnRNP K unfolding type-1245 i-motif DNA discretely revealed by single-molecule fluorescence resonance energy transfer (smFRET). **(A)** Schematic diagram of hnRNP K and Py25(1245)' i-motif DNA. **(B)** smFRET histograms obtained by adding 1 nM–1 μ M hnRNP K at series times from 0 to 10 min at pH 5.8. **(C)** Multipeak Gaussian distributions were used to fit the smFRET histograms at 10 min. The peak values are shown in the corresponding figures. **(D)** Transition density plots (TDPs) were used to show the state transitions of hnRNP K unfolding Py25(1245)' at 10 min and certain concentrations. **(E)** The fractions of the different folding structures at increasing concentrations of hnRNP K. UF, unfolded state; I1, intermediate state 1; I2, intermediate state 2; F, folded state.

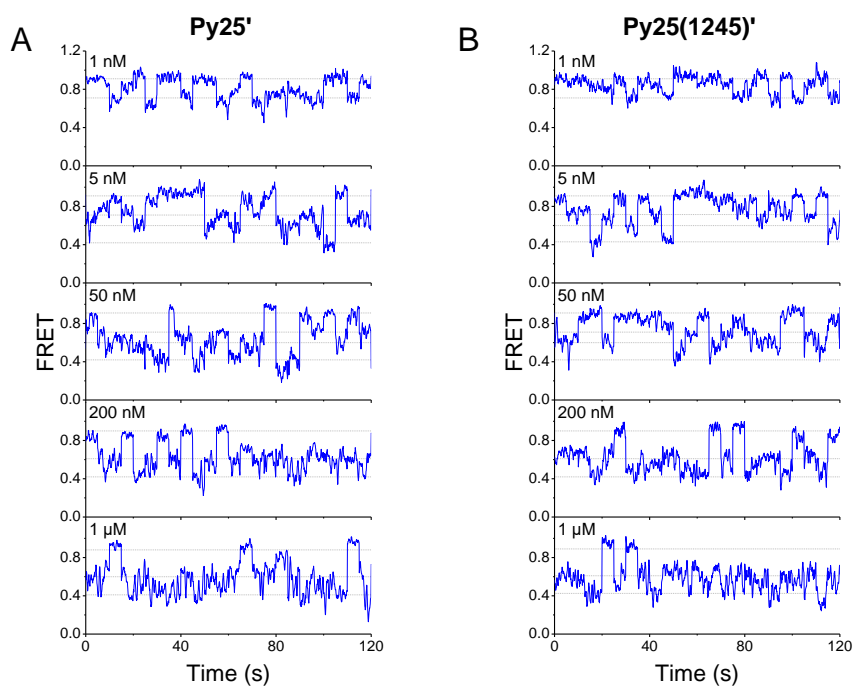


Figure S5. Representative single-molecule fluorescence resonance energy transfer (smFRET) traces of Py25' and Py25(1245)' measured at different concentrations of hnRNP K at pH 5.8.

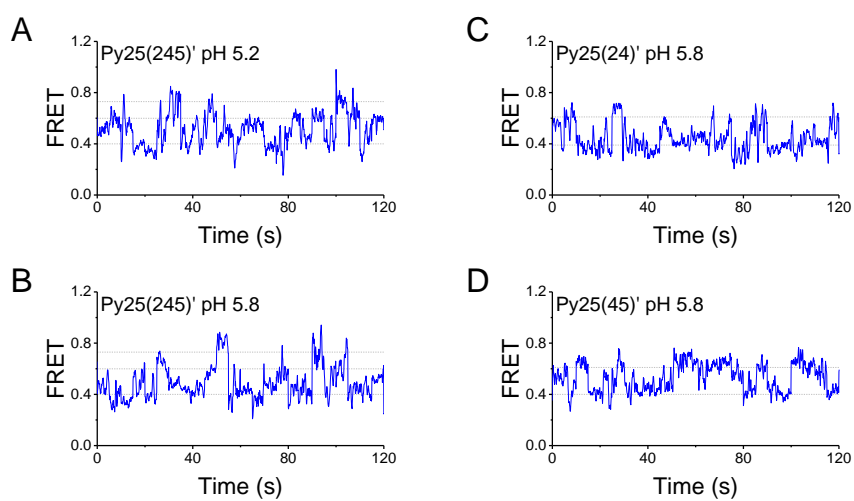


Figure S6. Representative single-molecule fluorescence resonance energy transfer (smFRET) traces of Py25(245)', Py25(24)', and Py25(45)' measured at corresponding pH values.

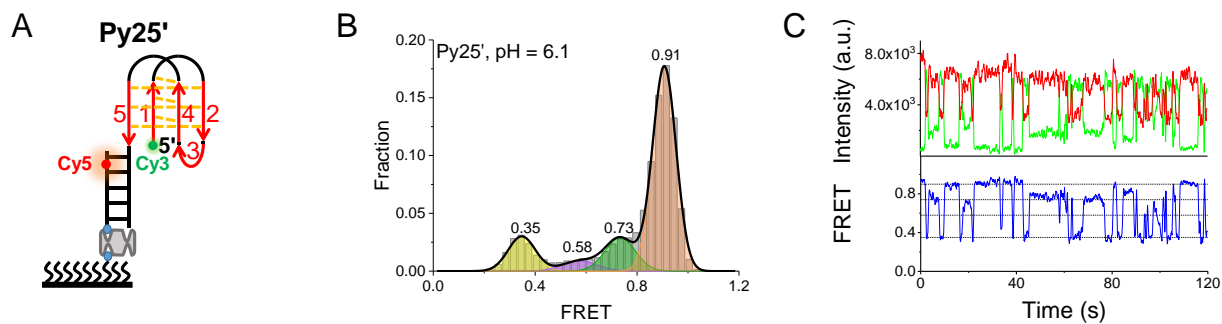


Figure S7. The folding of Py25' at pH 6.1. **(A)** Schematic diagram of Py25'. **(B)** FRET distributions of Py25' at pH 6.1. Multipeak Gaussian distributions were used to fit the smFRET histogram. **(C)** Representative time traces of fluorescence intensities of Cy3 and Cy5 (upper panel) and the corresponding FRET trace (lower panel).