Supporting material for:

Quantitative Analysis of Protein Unfolded State Energetics: Experimental and Computational Studies Demonstrate That Non-Native Side-Chain Interactions Stabilize Local Native Backbone Structure.

Junjie Zou[1,2], Shifeng Xiao[3], Carlos Simmerling[1,2,*], Daniel P. Raleigh[1,2,*]

[1]Department of Chemistry, Stony Brook University, Stony Brook, New York 11794-3400, United States

[2]Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, New York 11794-3400, United States

[3]Shenzhen Key Laboratory of Marine Biotechnology and Ecology, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China

* Authors to whom correspondence should be addressed:

DPR email: daniel.raleigh@stonybrook.edu phone: (631) 632-9547;

CS email: carlos.simmerling@stonybrook.edu, phone: (631) 632-5424.

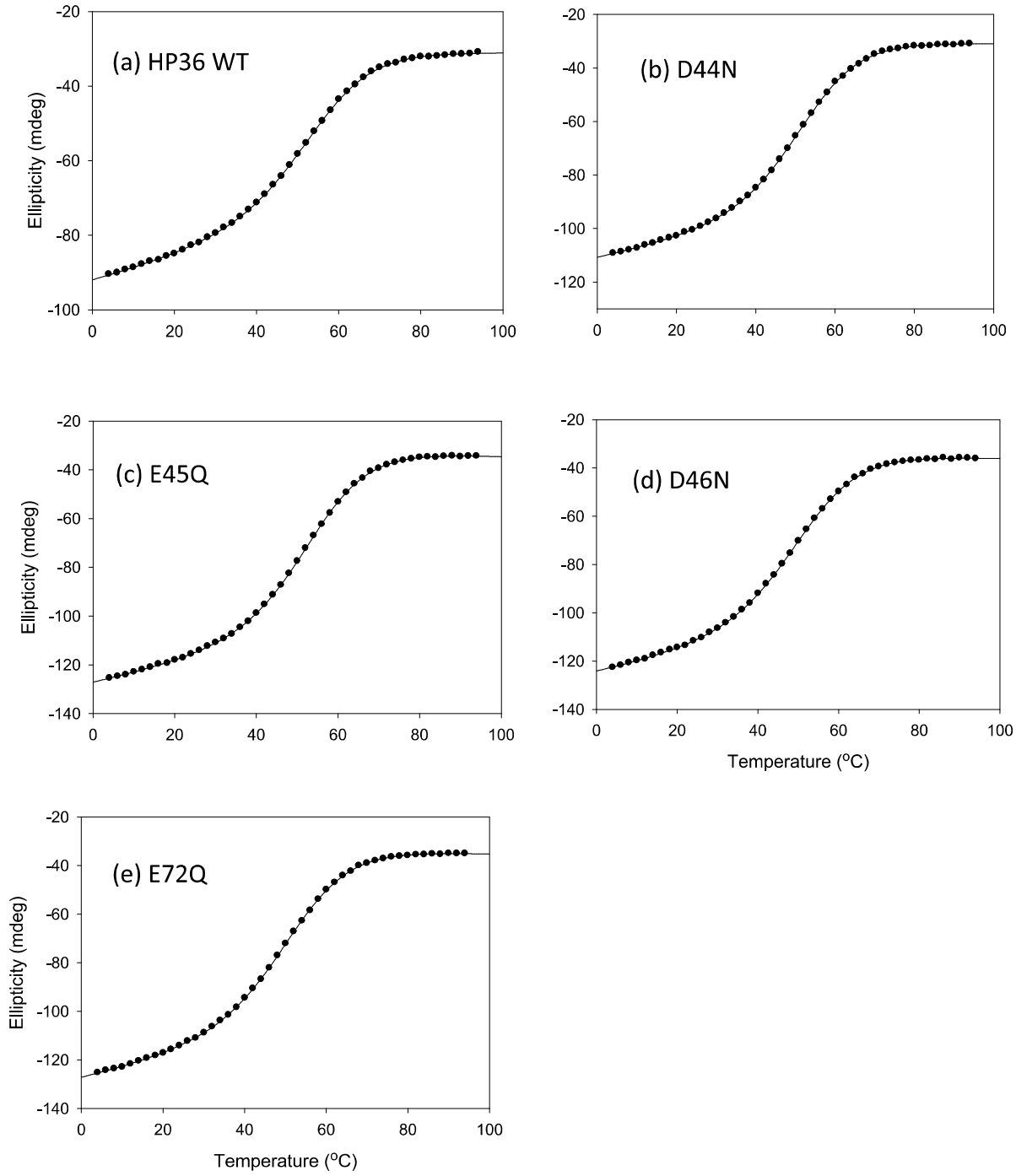

Figure S1. Temperature induced unfolding transitions of HP36 wildtype and mutants. (a)HP36 WT, (b)D44N, (c)E45Q, (d)D46N, (e)E72Q. The solid line represents the best fit to a two-state folding transition. Signals were recorded at 222 nm. All spectra were collected in 10 mM sodium acetate, 150 mM sodium chloride, pH 3.0 using a 1.0 cm cuvette.

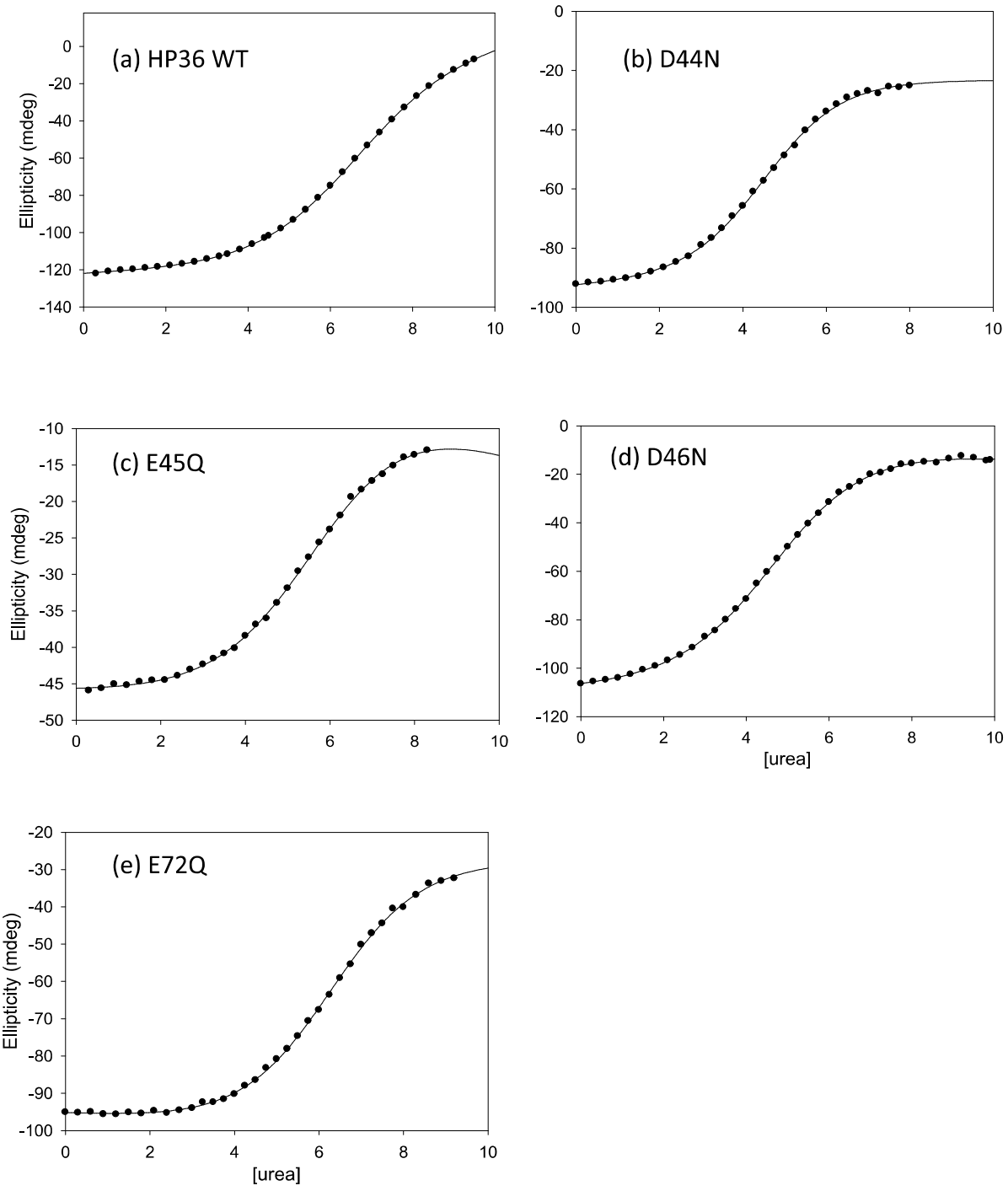

Figure S2. Urea induced unfolding transitions of HP36 wildtype and the mutants. (a)HP36 WT, (b)D44N, (c)E45Q, (d)D46N, (e)E72Q. The solid line represents the best fit to a two-state folding transition. Signals were recorded at 222 nm. All spectra were collected at 25 ^{o}C in 10 mM sodium acetate, 150 mM sodium chloride, pH 6.0 using a 1.0 cm cuvette.

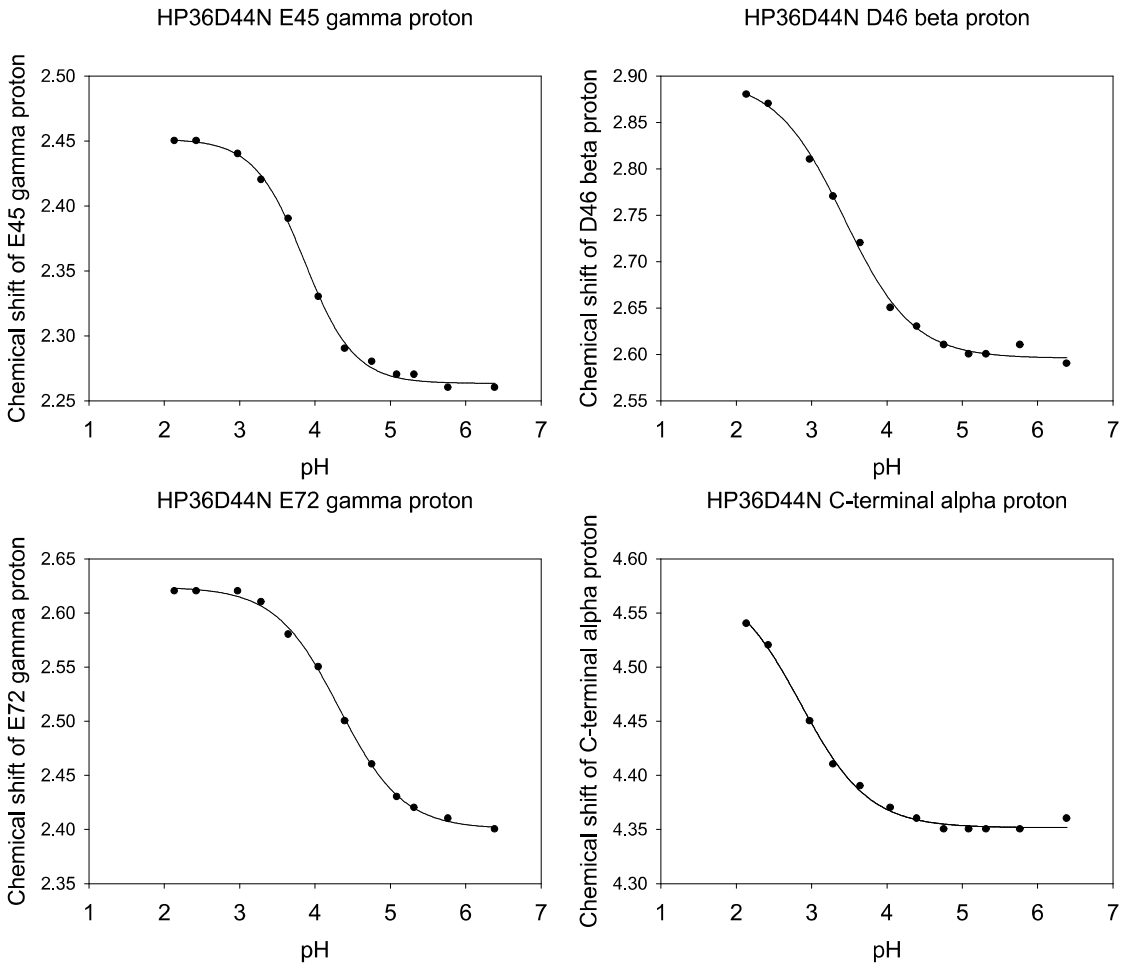

Figure S3. pKa determinations via a chemical shift monitored pH titration of native state of HP36D44N. The chemical shifts of γ-H, β-H and α-H protons were monitored for Glu, Asp and C-terminus respectively.

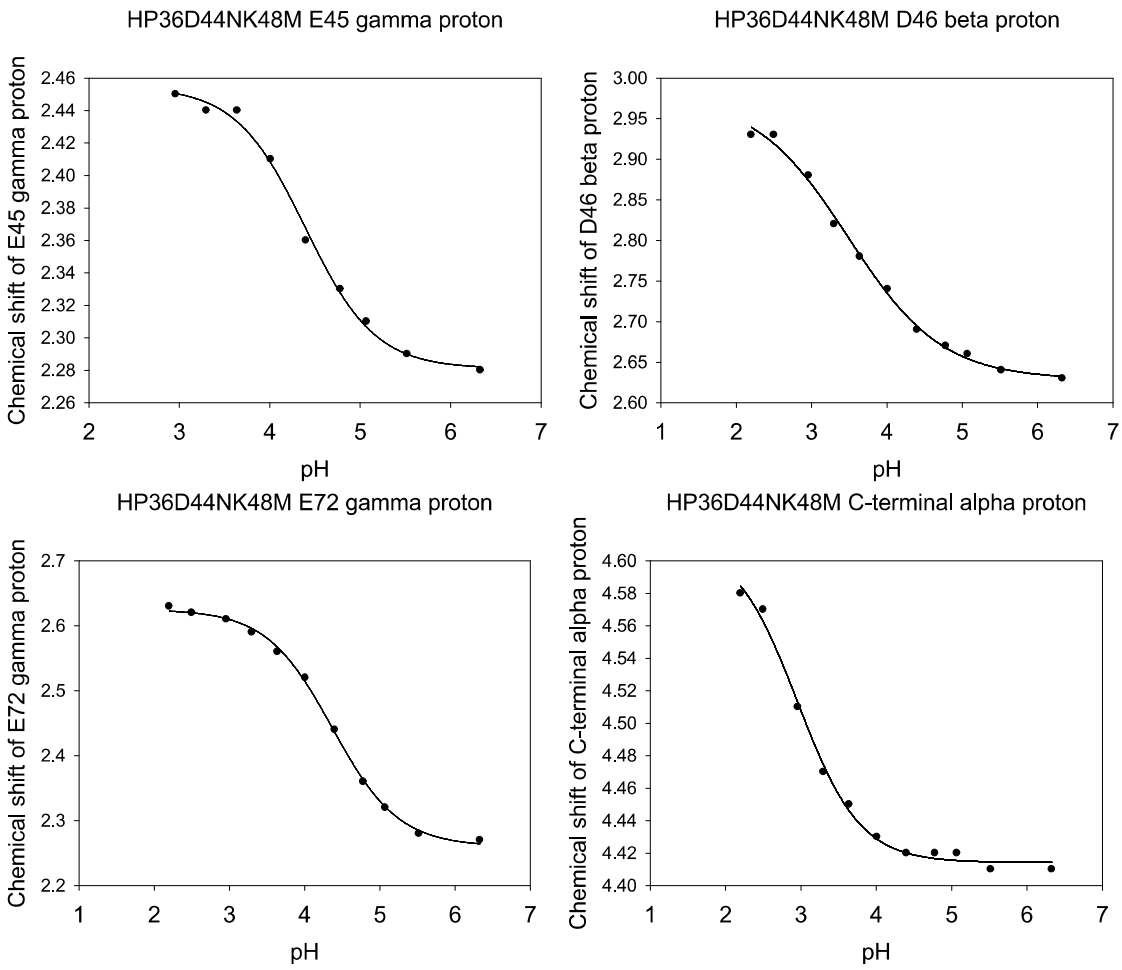

Figure S4. pKa determinations via a chemical shift monitored pH titration of native state of v. The chemical shifts of γ-H, β-H and α-H protons were monitored for Glu, Asp and C-terminus respectively.

Table S1. PDB atom name and atom type for an artificial Lys with dummy atoms mimicking Met using in the TI calculations

Amber PDB atom name for Lys with dummy atoms	Amber ff14sb atom type	
N	N	
H	H	
CA	CX	
HA	H1	
CB	2C	Same element but different atom type
HB2, HB3	HC	
CG	2C	Same element but different atom type
HG2, HG3	HC	
CD	S	Corresponds to SD of Met
HD2, HD3	DH	Dummy hydrogen
CE	CT	Same element but different atom type
HE2, HE3	H1	Same element but different atom type
NZ	H1	Corresponds to HE1 of Met
HZ1, HZ2, HZ3	DH	Dummy hydrogen
C	C	
O	O	

Table S2. PDB atom name and atom type for an artificial Asn with dummy atoms mimicking Asp using in the TI calculations

Amber PDB atom name for Lys with dummy atoms	Amber ff14sb atom type	
N	N	
H	H	
CA	CX	
HA	H1	
CB	2C	Same element but different atom type
HB2, HB3	HC	
CG	CO	Same element but different atom type
OD1	O2	Same element but different atom type
ND2	O2	Corresponds to OD2 in Asp
HD21, HD22	DH	Dummy hydrogen
C	C	
O	O	