Supplementary Material

Charge Effects in the Selection of NPF Motifs by the EH Domain of EHD1

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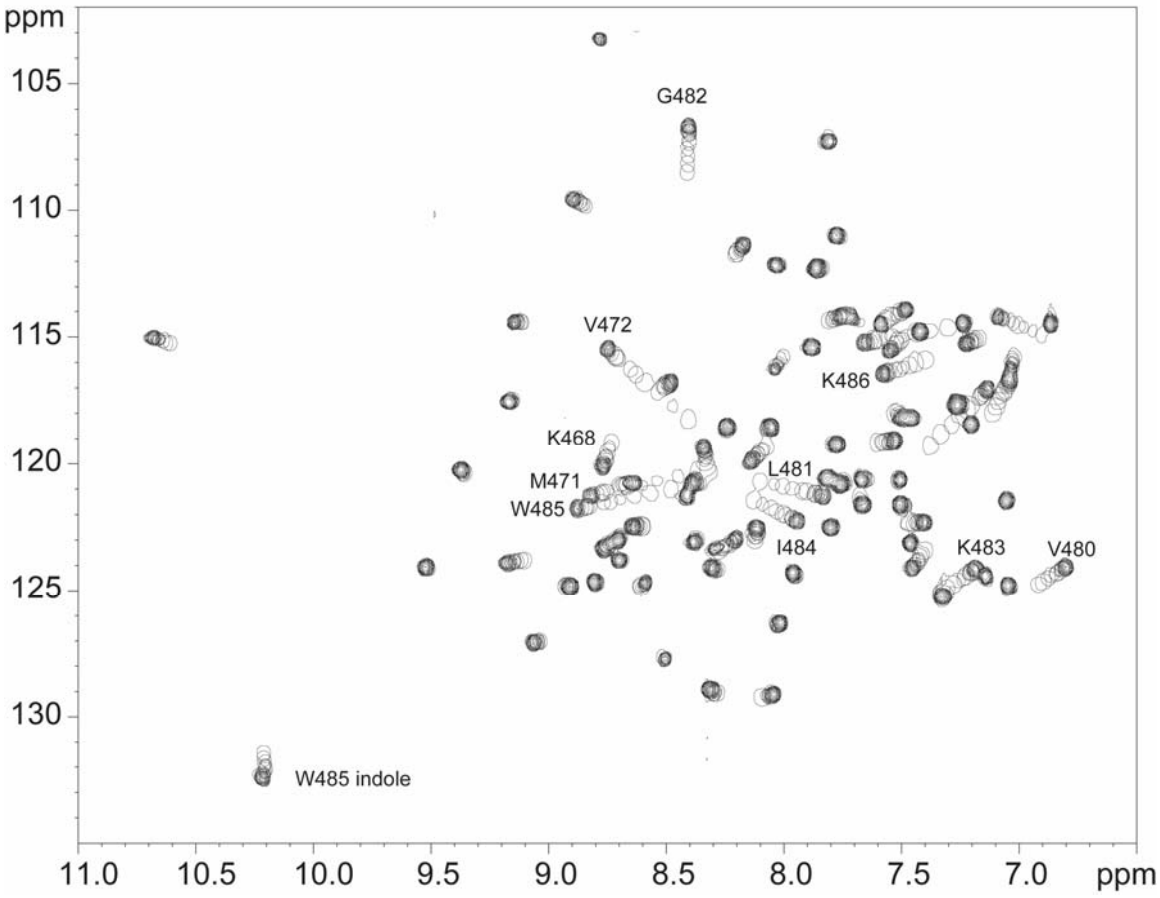

Figure S1. Overlaid $^{1}H/^{15}N$ HSQC spectra recorded at 500MHz (^{1}H) and 25˚C showing the titration of EHD1 EH with Rsn5-NPF1. Protein was 250 μM in 10 mM imidazole-d_4 pH 6.8, 10 mM NaCl, 1 mM $CaCl_2$, 0.02% NaN_3 and 10% D_2O. The final peptide concentration was 1.7mM. A single contour level is shown for each datapoint except the last. Selected residues that move substantially during the titration are labeled.

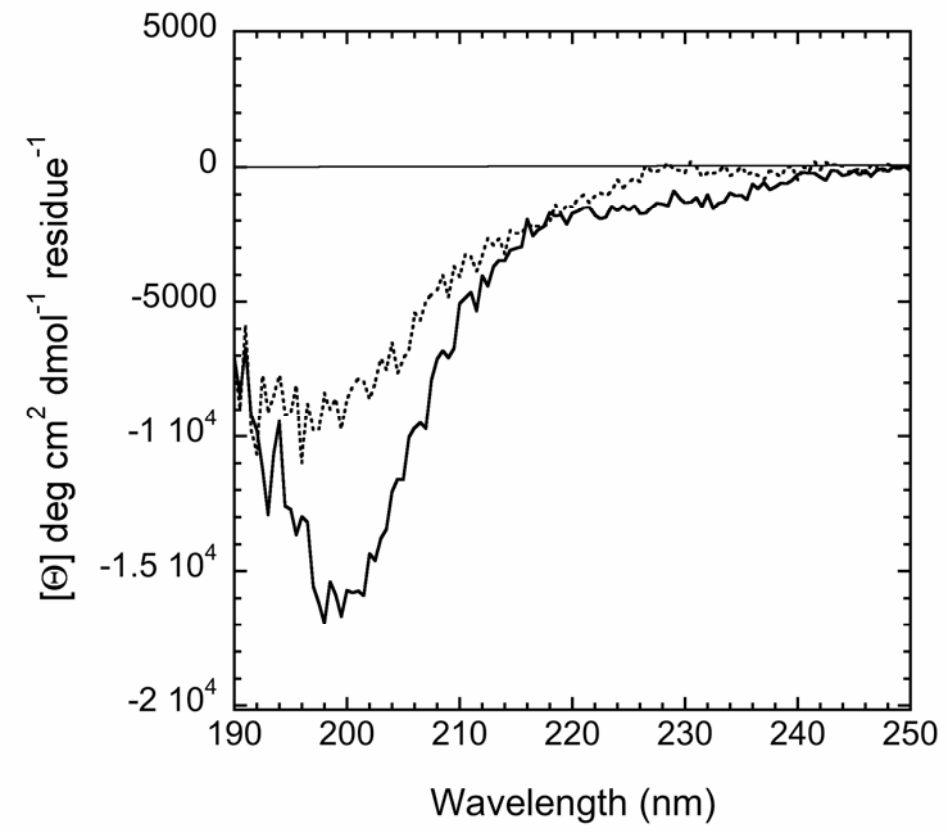

Figure S2. Far UV circular dichroism spectra of Rsn5-NPF1 (solid line) and Rfp-NPF2 (dotted line). Peptides were 0.1 mg mL^{-1} in 5 mM TrisHCl pH 7.5 and spectra were collected using a 1 mm pathlength cell at 5˚C. Neither peptide shows any evidence of β-turn formation and the spectra are consistent with random coil. The smaller absorption band at 200 nm for Rfp-NPF2 is expected due to positive contributions from the tyrosine sidechain at this wavelength (Brahms, S. and Brahms, J. 1980. *J. Mol. Biol.* **138** 149-178).

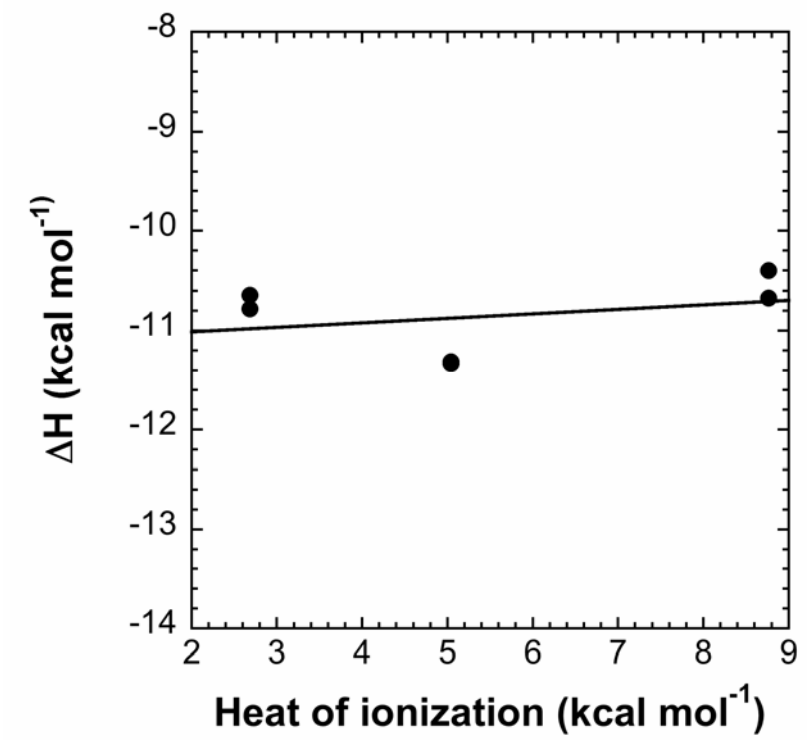

Figure S3. The apparent enthalpy of binding, $\Delta H_{apparent}$ is given by $\Delta H_{apparent} = \Delta H_{true} + n\ \Delta H_{ion}$, where ΔH_{true} is the true enthalpy of binding and n is the number of protons transferred to the buffer during the binding reaction. The plot shows the apparent enthalpy of binding of Rsn5-NPF1 to EHD1 EH as a function of buffer ionization enthalpy (ΔH_{ion}). The points represent titrations in 20mM PIPES (ΔH_{ion} = 2.68 kcal mol^{-1}), 20mM MOPS (ΔH_{ion} = 5.04 kcal mol^{-1}) and 20mM imidazole (ΔH_{ion} = 8.76 kcal mol^{-1}). All solutions contained 1mM $CaCl_2$. The slope, n, is 0.04, showing that proton transfer during the binding reaction is negligible.

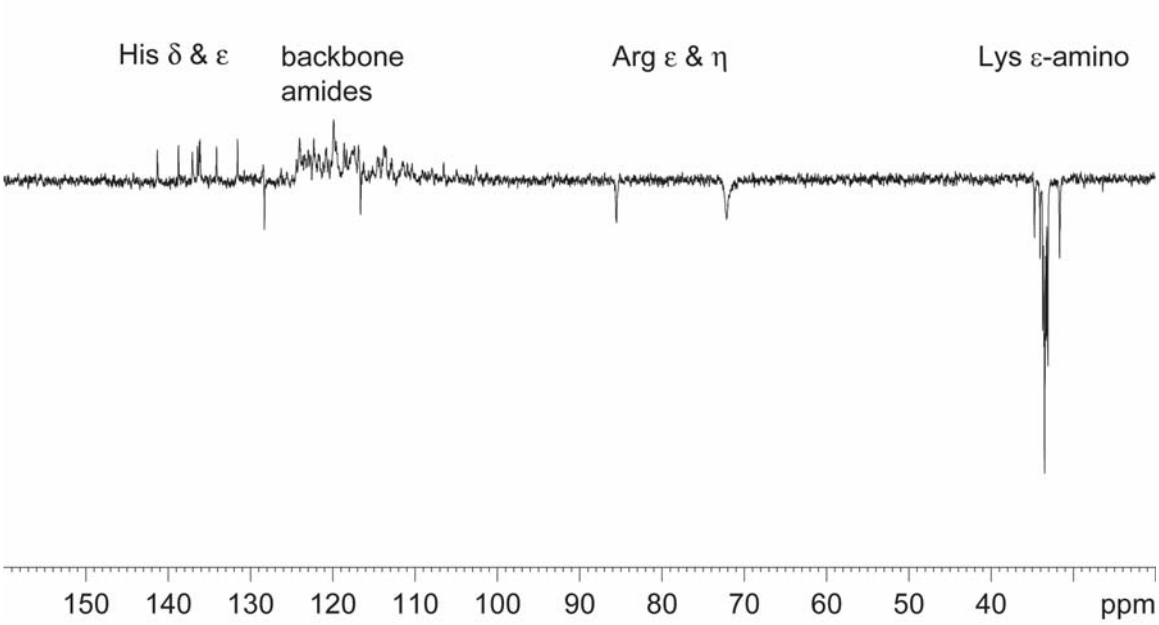

Figure S4. 30MHz proton-decoupled direct-observe ^{15}N spectrum of 2.5mM EHD1 EH without peptide at 25˚C. 20,000 scans were collected with a 2.7s acquisition time and 0.1s recycle delay with WALTZ decoupling of the protons applied continuously. The spectral width was 200 ppm. Line broadening is 1Hz. ^{15}N chemical shifts are reported with respect to liquid ammonia at 0 ppm. The large negative ^{15}N NOE results in negative peaks for the more mobile nitrogen atoms.

Table S1. Association constants for other NPF peptide-EH protein interactions.

Peptide		Protein	K_a (M^{-1})
Ac-LKEYNPFEEED-NH_2	Rsn5-NPF2	EHD1 EH	9.78e+5
Ac-AVAGNPFIQPD-NH_2	Rsn5-NPF3	EHD1 EH	1.40e+4
Ac-SPAPNPFSEED-NH_2	Rsn5-NPF4	EHD1 EH	1.11e+5
Ac-AVAGNPFIQED-NH_2		EHD1 EH	6.39e+4
Ac-AVAGNPFIEED-NH_2		EHD1 EH	2.14e+5
Ac-AVAGNPFDEED-NH_2		EHD1 EH	6.65e+5
Ac-GPSLNPFDEED-NH_2	Rsn5-NPF1	Reps1 EH	2e+3
Ac-GPSLNPFDEED-NH_2	Rsn5-NPF1	Eps15 EH_2	no measurable binding
Ac-YESTNPFTAK-NH_2	Rfp2-NPF2	Reps1 EH	2e+3