Electronic Supplementary Material (ESI) for Molecular BioSystems.

Histidine switch controlling pH-dependent protein folding and DNA binding in a transcription factor at the core of synthetic network devices

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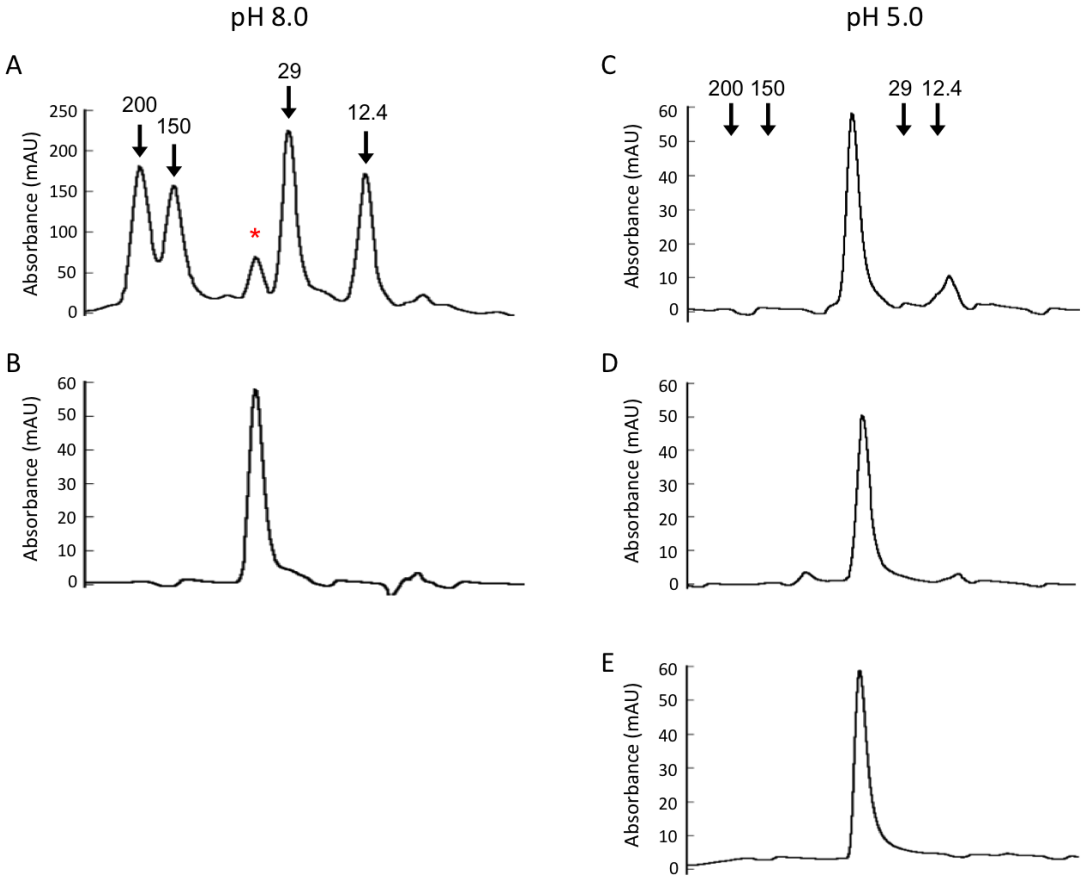


Figure S1. Gel filtration chromatography. A. Gel filtration standard elution profile at pH 8.0. Standards are identified by their Mw (in kDa). The peak marked with red asterisk is HucR-WT (calculated Mw of monomer 19.7 kDa). B. HucR-WT at pH 8.0. C. HucR-WT at pH 5.0. Arrows correspond to elution of gel filtration standards. The minor species eluting at a MW~6 kDa is likely due to protein interacting with the column matrix. D. HucR-E48Q at pH 5.0. D. HucR-H51F at pH 5.0.

5'-GCGGCCCTCAGTAGGTAGACATCTAAGTATCTGGCGGCGG-3'

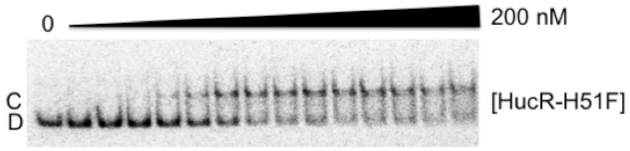


Figure S2. Electrophoretic mobility shift assay at room temperature of HucR-H51F at pH 5.0. Complex (C) and free DNA (D) are indicated at the left. The sequence of 40 bp DNA used in thermal stability assays shown above the gels with the imperfect palindrome representing the HucR binding site shown in red. The 77 bp DNA used in EMSA contained the cognate site embedded in longer flanking sequence.

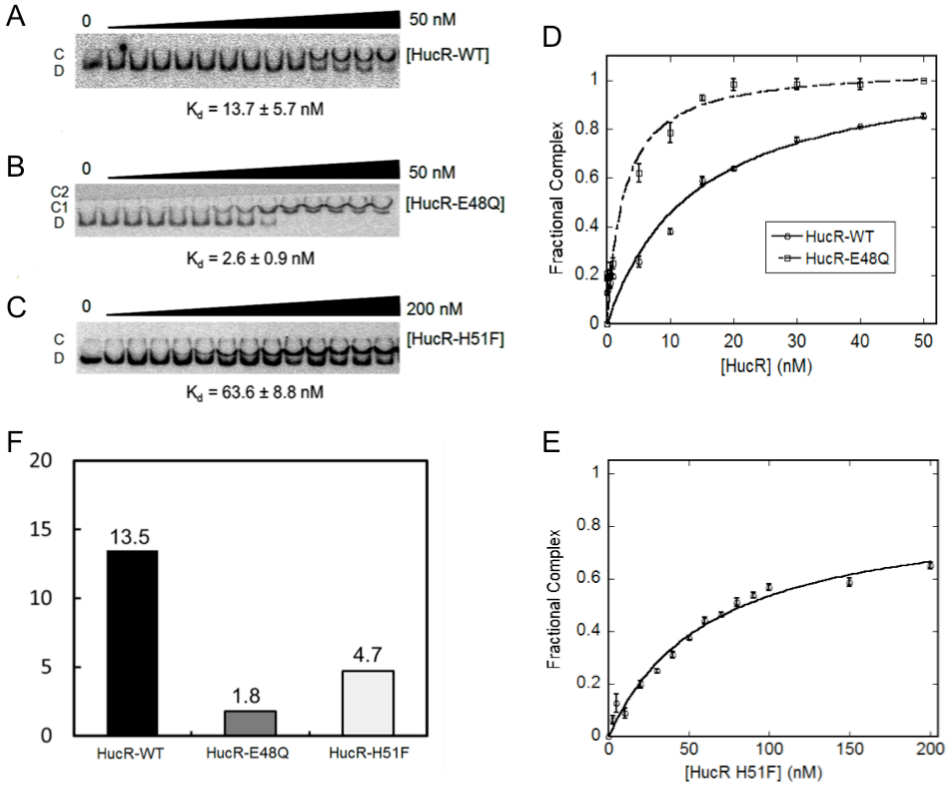


Figure S3. Electrophoretic mobility shift assay at pH 5.0 performed at 4 °C. A-C. HucR-WT, HucR-E48Q, and HucR-H51F binding to 0.1 nM *hucO* (77-bp). Complex (C) and free DNA (D) are indicated at the left. D. Binding isotherm for HucR-WT (continuous line) and HucR-E48Q (broken line). E. Binding isotherm for HucR-H51F. Error bars represent standard deviation from three independent experiments. F. Fold increase in dissociation constant (K_d) at pH 5.0 (4 °C) compared to pH 8.0 (room temperature).

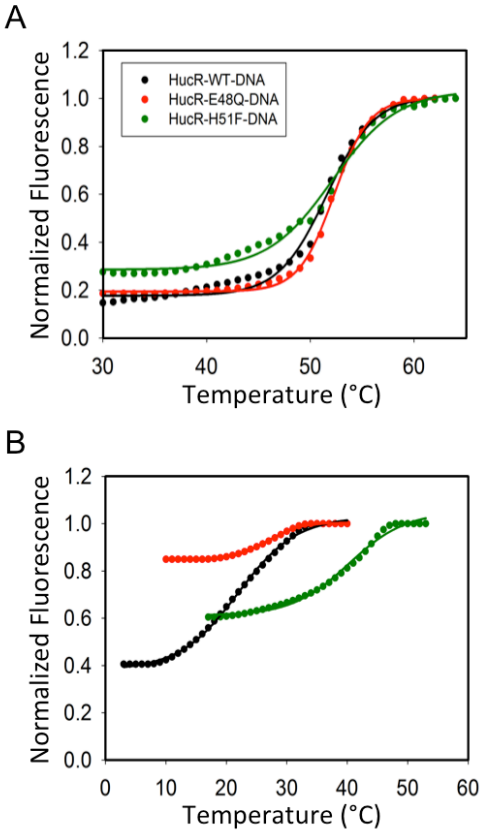


Figure S4. Thermal denaturation of proteins in complex with DNA. Normalized fluorescence of SYPRO Orange as a function of temperature for HucR-WT (black), HucR-E48Q (red), and HucR-H51F (green). A. pH 8.0. B. pH 5.0.

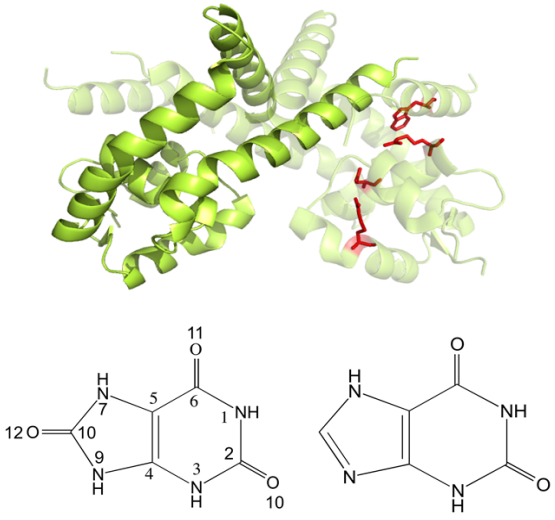
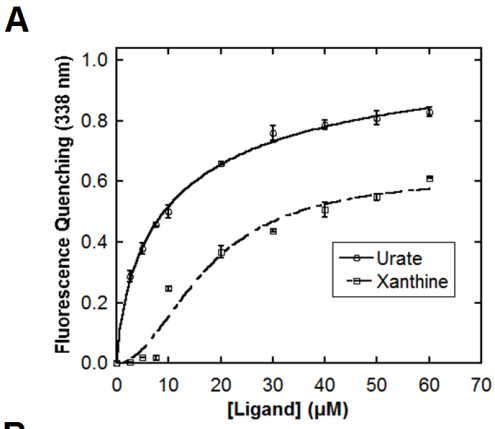
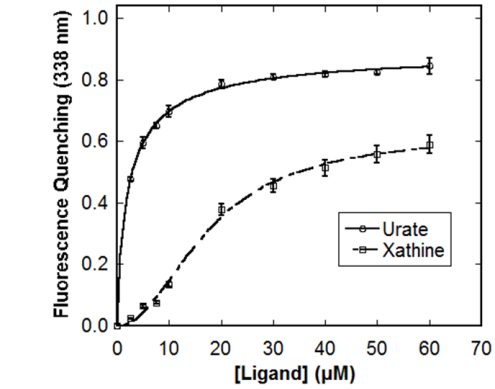


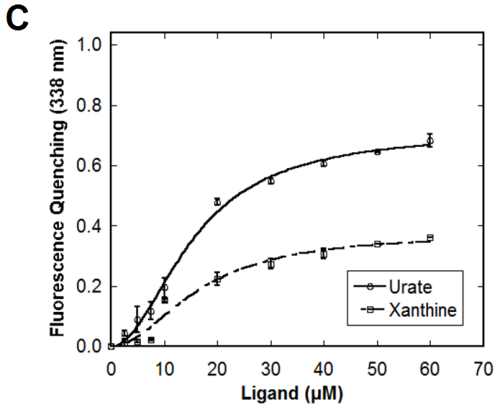
Figure S5. HucR ligand binding site. Top panel shows structure of HucR-WT (2fbk) with predicted ligand-binding residues in red stick representation. Ligands urate (left) and xanthine shown below the structure.



	Urate	Xanthine
	K_d = 11.4 ± 2.9 μM	K_d = 17.2 ± 3.7 μM
	n_H = 0.7 ± 0.1	n_H = 2.0 ± 0.5



	Urate	Xanthine
	K_d = 2.2 ± 0.8 μM	K_d = 17.6 ± 1.3 μM
	n_H = 0.8 ± 0.0	n_H = 2.0 ± 0.2



	Urate	Xanthine
	K_d = 14.7 ± 1.2 μM	K_d = 16.2 ± 2.7 μM
	n_H = 2.1 ± 0.2	n_H = 1.8 ± 0.3

Figure S6. Intrinsic tryptophan fluorescence quenching at 338 nm as a function of increasing concentration of urate (solid line) and xanthine (broken line). A. HucR-WT. B. HucR-E48Q. C. HucR-H51F. Corresponding K_d and n_H are indicated at the right.

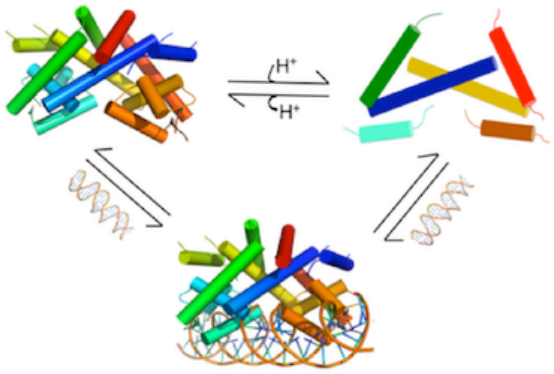


Figure S7. Conformational changes in HucR. Protonation of H51 reversibly converts compactly folded HucR into a molten globule state. DNA binding restores the compact folded state.

Table S1. Secondary structure content determined from CD spectra

	pH 7.0			pH 5.0		
Secondary Structure	HucR-WT	HucR-E48Q	HucR-H51F	HucR-WT	HucR-E48Q	HucR-H51F
α-Helix	64%	55%	63%	67%	64%	62%
β-Sheets	12%	16%	16%	13%	13%	13%
Turns	9%	7%	7%	5%	6%	13%
Unordered	15%	22%	14%	14%	17%	12%

Table S2. Effect of urate (μM) on thermal stability (T_m in °C)

Protein	pH 8.0	Urate (10)	Urate (50)	Urate (100)
HucR-WT	51.6 ± 0.4	41.6 ± 0.2 52.6 ± 0.3	49.7 ± 0.4	48.8 ± 0.2
HucR-E48Q	52.2 ± 0.1	n/a[a]	53.1 ± 0.2	53.4 ± 0.2
HucR-H51F	52.7 ± 0.1	n/a[a]	45.6 ± 0.1	41.9 ± 0.2

[a]Insufficiently distinct transitions to estimate individual Tm values. For 50 and 100 μM urate, estimation of T_m assumed a one-step transition. Values determined based on SYPRO Orange fluorescence ± SD.

Table S3. Data collection and refinement statistics for crystal structure of HucR-E48Q

	HucR-E48Q
Data collection	
Space group	P 61
Cell dimensions	
a,*b*,*c* (Å)	44.931 44.931 284.825
a, b, g (Å)	90 90 120
Wavelength	0.979200
Resolution range	38.91 - 2.052 (2.125 - 2.052)
Completeness (%)	0.99
Wilson B-factor	41.18
Refinement	
R-work / R-free	0.2169/0.2567 (0.2998/0.3298)
No. atoms	
Protein	2386
Ligands/ion	4
B-factor (Å^2)	
Protein	63.67
Ligands/ion	48.83
Solvent	50.09
R.m.s deviations	
RMS (bonds)	0.015
RMS (angles)	1.74
Ramachandran (%)	
Most favored	97
Additionally allowed	2.7
Outliers	0.67

[1]Values in parentheses are for highest resolution shell.

Supplemental Experimental Procedures

Preparation of HucR mutant proteins

Residues in the HucR dimer interface were mutated by whole-plasmid PCR using as template the recombinant plasmid, which contains the gene encoding *D. radiodurans* HucR without any tags. His51 was substituted with Phe using primers H51F-Fwd 5′-CTGGGGCGTGAAATCGAGCGGA-3′ and H51F-Rev 5′-AGCCGC**GAA**CAGCCGCTC-3′ while Glu48 was mutated to Gln using E48Q-Fwd 5′-GCTGCTC**CAG**CGGCTGC-3′ and E48Q-Rev 5′-AGCAGGGTCAGCATCGGCC-3′ (mutated codons in boldface). The PCR reactions were treated with DpnI and transformed into *E. coli* NovaBlue electrocompetent cells. The fidelity of constructs were confirmed by DNA sequencing. The verified plasmids were transformed into *E. coli* BL21(DE3)pLysS for overexpression of proteins.

HucR-E48Q was purified according to the previously reported protocol for HucR, whereas the HucR-H51F purification method was modified. HucR-H51F was overexpressed by growing cells in Luria Broth containing 100 μg/ml ampicillin and 35 μg/ml chloramphenicol to an optical density at 600 nm of 0.5; protein expression was then induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside for 1h. The cells were pelleted by centrifugation and then resuspended in lysis buffer (50 mM Tris-HCl pH 8.0, 25 mM NaCl, 5% glycerol (v/v), 5 mM EDTA, 0.15 mM phenyl methyl sulfonylfuoride (PMSF), and 10 mM 2-mercaptoethanol) and lysed by addition of 200 μg/ml lysozyme for 1h. The clarified lysate was loaded onto tandem CM-sepharose and DEAE-cellulose columns equilibrated with HA buffer (20 mM Tris-HCl pH 8.7, 50 mM KCl, 4.8% glycerol (v/v), 1 mM EDTA, 0.2 PMSF, and 4.3 mM 2-mercaptoethanol). The flow-through was collected and dialyzed against HAP-A buffer (20 mM potassium phosphate pH 7.0, 50 mM KCl, 4.8 % glycerol (v/v), 1 mM EDTA, 4.3 mM 2-mercaptoethanol, 0.2 mM PMSF) and applied to a hydroxyapatite-cellulose column equilibrated with the same buffer. The flow-through containing HucR-H51F was loaded on to a heparin-agarose column equilibrated with HAP-A buffer. The protein was eluted by a linear gradient of KCl from 50 mM to 1M. The fractions containing protein were pooled and dialyzed against HAP-A buffer and loaded on to a second heparin-agarose column at pH 8.0 and eluted with linear gradient of 50 mM to 1M KCl. Fractions containing purified HucR-H51F were collected, concentrated and stored at -80 ºC after raising the glycerol concentration to 20%. The purity of protein was verified by SDS-PAGE, and the concentration determined by Micro BCA Protein Assay Kit (Pierce) using bovine serum albumin (BSA) as standard.