A new role for Zinc limitation in bacterial pathogenicity: modulation of α-hemolysin from uropathogenic *Escherichia coli*.

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Supplementary information

Table S1. Primers used in this study.

HlyA-P1	5'- ATCATATCCATTTTCAAAGTAATTTTTGCCGTGTTTTGTG TAGGCTGGAGCTGCTTC -3'
HlyA-P2	5'- CAAATTAAAAGCACACACTGCAGTCTGCAAAGCAATCCG CATATGAATATCCTCCTTAGT -3'
Hly1-P1	5'- CCTGATTAATTCAACCCCTTATCTGACAGGGGTAGAAGT GTAGGCTGGAGCTGCTTC -3'
Hly1-P2-2	5'- ATTCAGCAATAACATCGGCCATTTTACTGGCGACATGCA TATGAATATCCTCCTTAGT -3'
Zur-P1	5'- CCATGTGCTTCAATAACATTATGCCGCAGGGCAAAACCC ATTGTGTAGGCTGGAGCTGCTTCG -3'
Zur-P2	5'- CACCCTGTTCTCGGCGTTTAAGTGAGAACTATGGTAAAG TCATATGAATATCCTCCTTA -3'
Zur-P1.2	5' – TTACCGCTGGTAATTAATCCCTCCTGCCCGACATGTAC AAGGGTGTAGGCTGGAGCTTCG -3'
Zur-P2.1	5'- GCTACTATAGCATAACGAATATGCGATCAGGCAATGTGAC CATATGAATATCCTCCTTAGT -3'
526	5'- CTGTATGGAACGGGATG -3'
HlyII-BamIII	5'- GGATCCATGCCCAAGAACCTC -3'
HlyII-GSP2	5'- TAAAAAGATCACATAAAGAAATGG -3'
HlyII-GSP3	5'- CCTGCACCTTGCTTCATTTG -3'
Zur-*Bam*HI	5'- GGATCCCATCGGGGCAATCGAGAAAC -3'
Zur-*Eco*RI	5'- GAATTCCAGGAACGCTACGGTTATC -3'

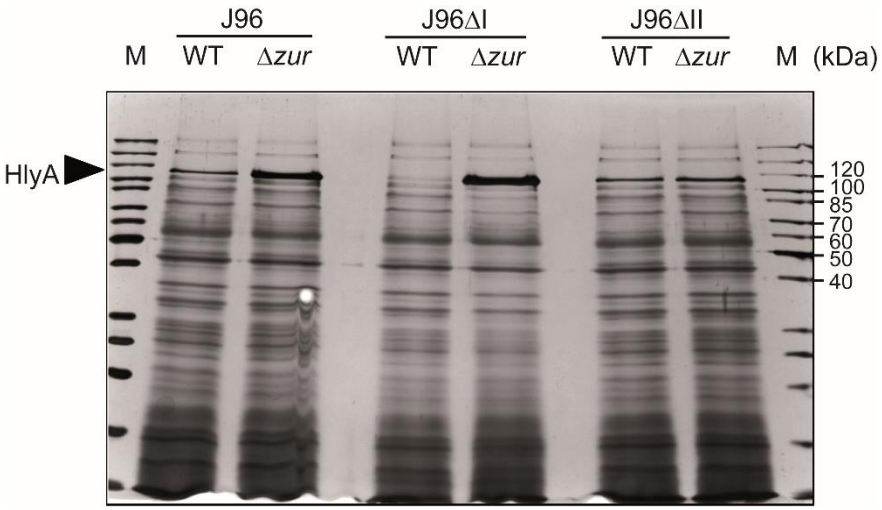


Figure S1. **Electrophoretic analysis of secreted protein extracts of J96, JFV16 (J96ΔII) and JFV21 (J96ΔI)**. Bacterial cultures were grown in LB at 37 ºC up to late-log phase (OD_{600nm} of 1.0). Secreted extracts were analyzed in silver stained 10 % SDS-PAGE. The band corresponding to HlyA is indicated with an arrowhead. Lanes M: molecular mass markers (size indicated in kDa).

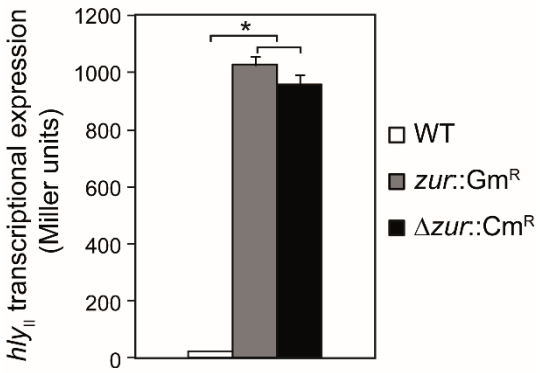


Figure S2. *zur* mutation causes derepression of the *hly$_{II}$* operon expression in cultures grown up to mid-log phase. Transcriptional expression of the *hly$_{II}$* operon from cultures of the JFV3 strain (J96 *hlyII::lacZ*) and its *zur*::Gm^R and *zur*::Cm^R derivatives. Cultures were grown in LB at 37ºC up to mid-log phase (OD_{600nm} of 0.4). β-galactosidase activity (Miller units) was determined in three independent cultures. Mean values with standard deviations are plotted. * $P < 0.05$, ANOVA with Tukey's multiple comparisons test.

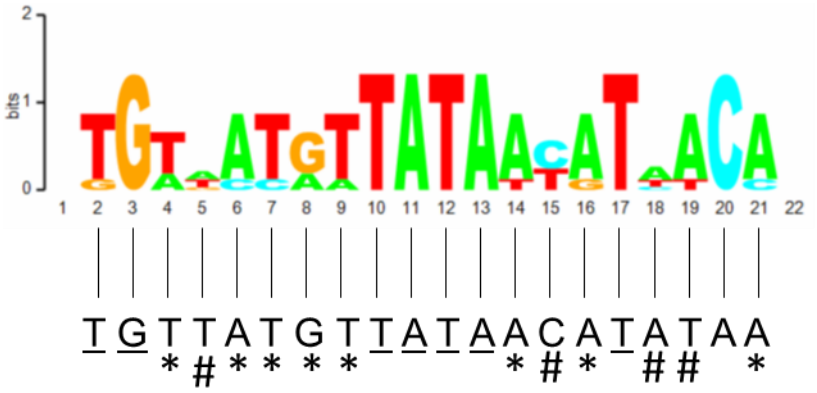


Figure S3. Alignment of the *hly$_{II}$* Zur binding site to the Zur consensus sequence[1]. The *hly$_{II}$* Zur binding site was aligned with the sequence logo representation of the template strain in Zur-DNA recognition based on the following *E. coli* Zur operators: *znuA*, *znuC*, *zinT*, and *l31p*. Underlined bases indicate identical to the consensus residues, bases with * are identical to the most predominant residue and bases labelled with # are identical with no predominant residues.

1 Gilston, B. A. *et al.* Structural and Mechanistic Basis of Zinc Regulation Across the E. coli Zur Regulon. *PLoS Biol.* **12,** e1001987 (2014).

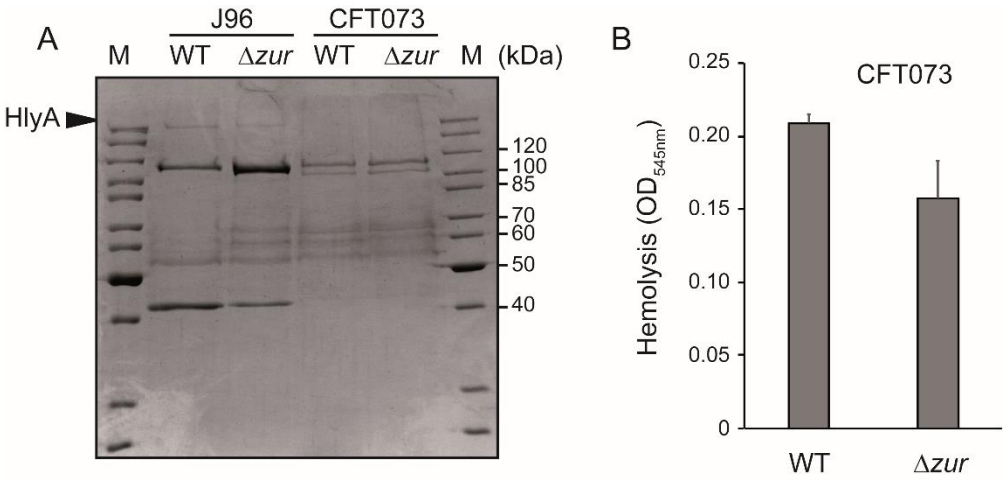


Figure S4. Effect of Δ*zur* mutation in the expression of α-hemolysin in the UPEC strain CFT073. A. Electrophoretic analysis of secreted protein extracts of J96, EV27 (J96Δ*zur*), CFT073 and DJ1 (CFT073Δ*zur*). Bacterial cultures were grown in LB at 37 ºC up to late-log phase (OD_{600nm} of 1.0). Secreted extracts were analyzed in Coomassie blue stained 10 % SDS-PAGE. The band corresponding to HlyA is indicated with an arrowhead. Lanes M: molecular mass markers (size indicated in kDa). B. Hemolysis after 1 hour infection of sheep blood cell suspensions with CFT073 and DJ1 (CFT073Δ*zur*). The release of haemoglobin was monitored by OD_{545nm}.

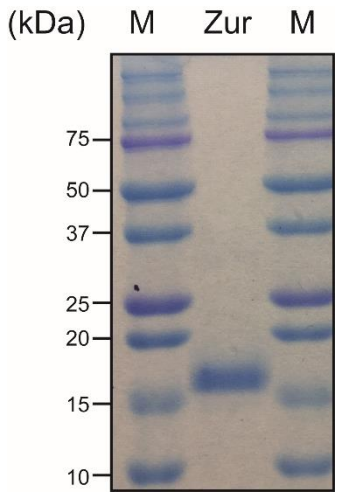
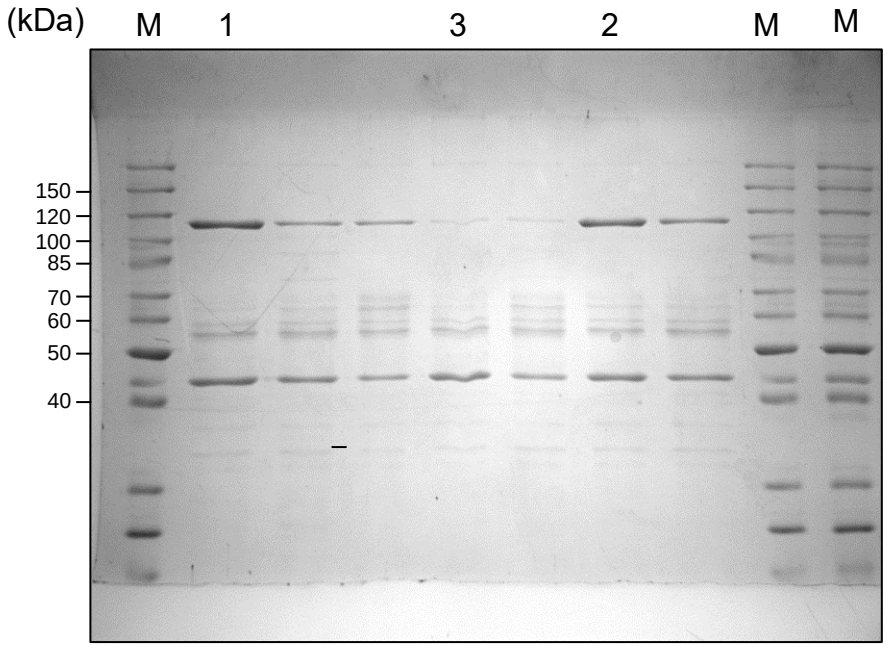


Figure S5. Pure Zur protein. 5 μg of pure Zur protein was analysed in 12% SDS-PAGE, stained by SimpleBlue safe stain (Invitrogen). Lanes M: molecular mass markers (size indicated in kDa).

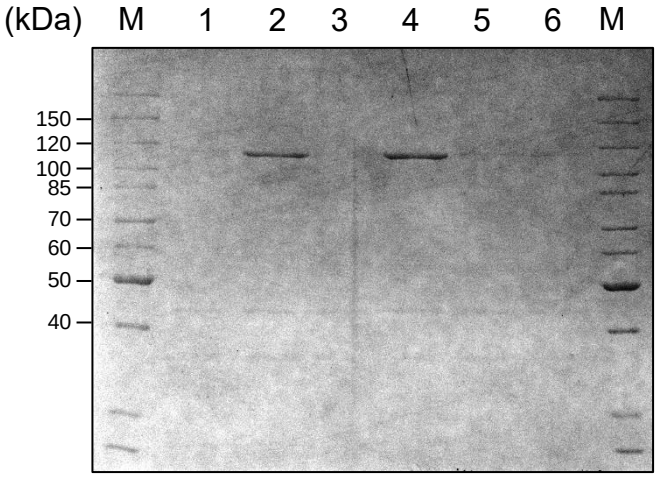
Coomassie stained gel



Lane 1: Cell-free extract from J96
Lane 2: Cell-free extract from J96ΔII
Lane 3: Cell-free extract from J96ΔI
Lanes M: molecular mass markers (size indicated in kDa).

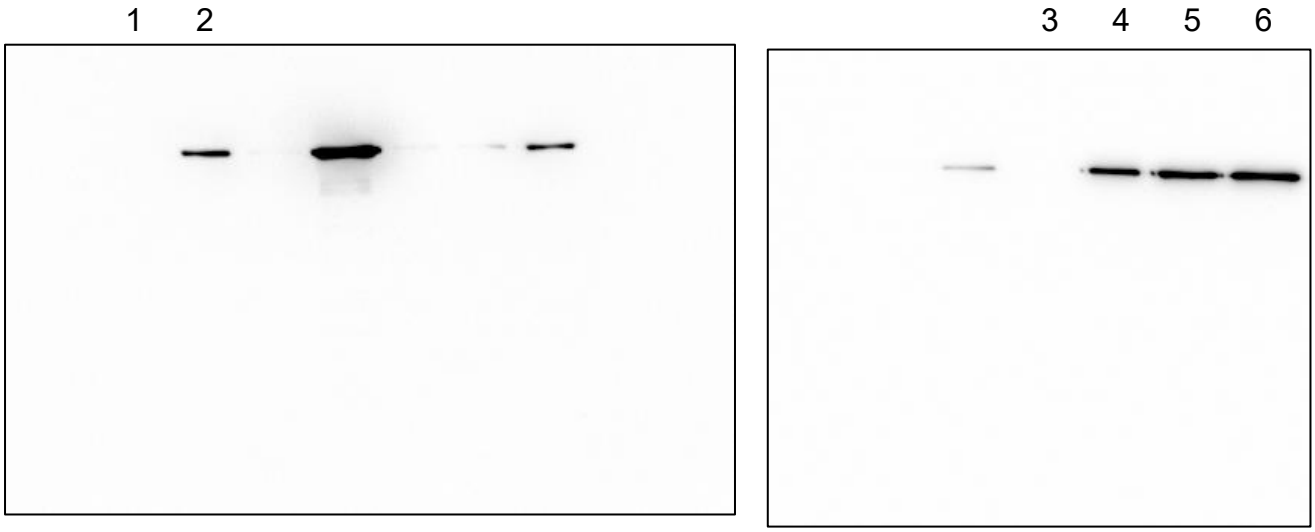
Figure S6. Original gel image used to create Fig. 1c panel.

Coomassie stained gel



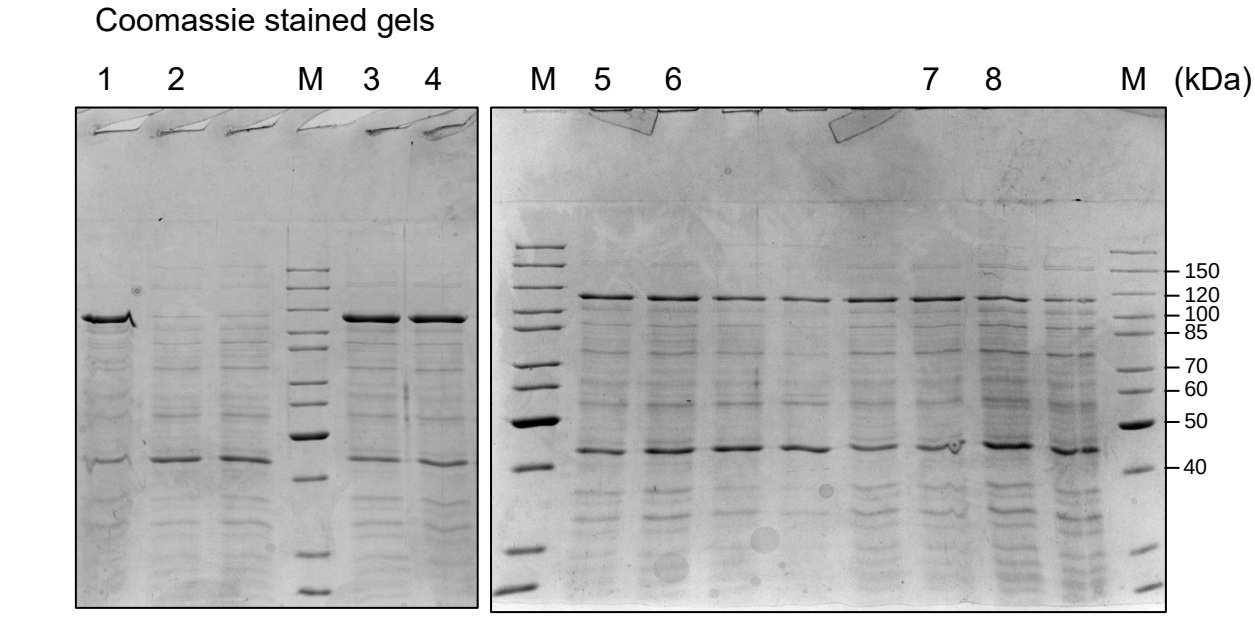
Lane 1: Cell-free extract from J96
Lane 2: Cell-free extract from J96Δzur
Lane 3: Cell-free extract from J96ΔI
Lane 4: Cell-free extract from J96ΔIΔzur
Lane 5: Cell-free extract from J96ΔII
Lane 6: Cell-free extract from J96ΔIIΔzur
Lanes M: molecular mass markers
(size indicated in kDa).

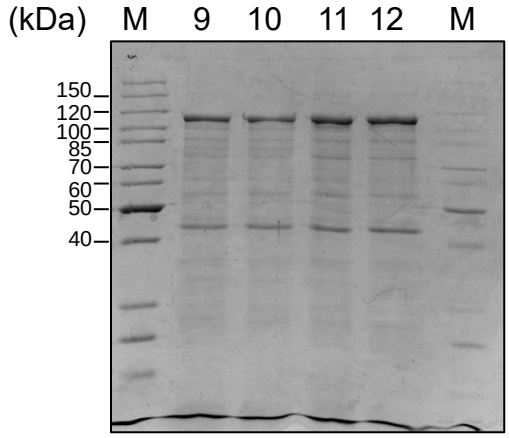
Western blots anti-HlyA



Lane 1: Cell-free extract from J96
Lane 2: Cell-free extract from J96Δzur
Lane 3: Cell-free extract from J96ΔI
Lane 4: Cell-free extract from J96ΔIΔzur
Lane 5: Cell-free extract from J96ΔII
Lane 6: Cell-free extract from J96ΔIIΔzur

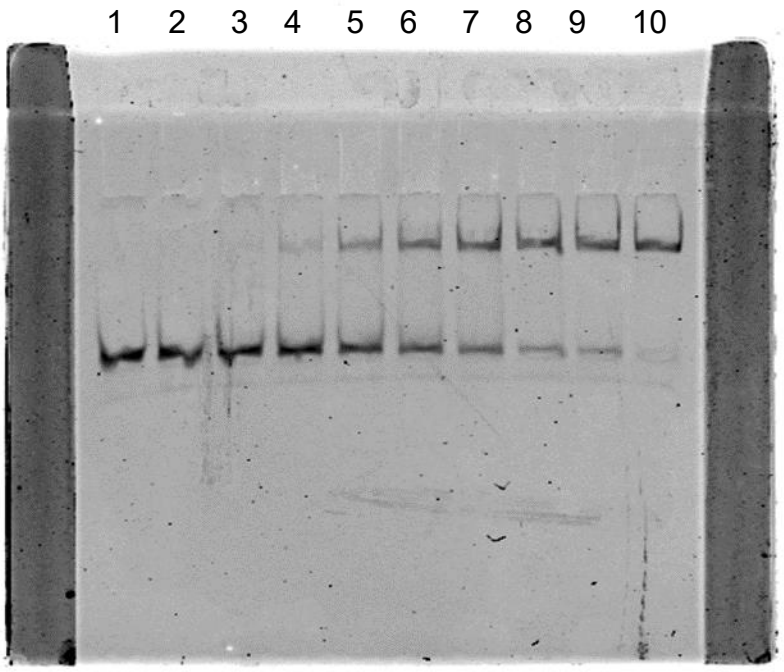
Figure S7. Original gel and blot images used to create Fig. 2c panel.





Lane 1: Cell-free extract from J96ΔI (- $ZnCl_2$)
Lane 2: Cell-free extract from J96ΔI (+ $ZnCl_2$)
Lane 3: Cell-free extract from J96ΔIΔzur (- $ZnCl_2$)
Lane 4: Cell-free extract from J96ΔIΔzur (+ $ZnCl_2$)
Lane 5: Cell-free extract from J96ΔII (- $ZnCl_2$)
Lane 6: Cell-free extract from J96ΔII (+ $ZnCl_2$)
Lane 7: Cell-free extract from J96ΔIIΔzur (- $ZnCl_2$)
Lane 8: Cell-free extract from J96ΔIIΔzur (+ $ZnCl_2$)
Lane 9: Cell-free extract from J96 (- $ZnCl_2$)
Lane 10: Cell-free extract from J96 (+ $ZnCl_2$)
Lane 11: Cell-free extract from J96Δzur (- $ZnCl_2$)
Lane 12: Cell-free extract from J96Δzur (+ $ZnCl_2$)
Lanes M: molecular mass markers (size indicated in kDa).

Figure S8. Original gel images used to create Fig. 4c panel.



Lane1: absence of Zur

Lane 2: 0.015 nM Zur dimer

Lane 3: 0.03 nM Zur dimer

Lane 4: 0.05 nM Zur dimer

Lane 5: 0.1 nM Zur dimer

Lane 6: 0.15 nM Zur dimer

Lane 7: 0.2 nM Zur dimer

Lane 8: 0.3 nM Zur dimer

Lane 9: 0.35 nM Zur dimer

Lane 10: 0.9 nM Zur dimer

Figure S9. Original gel image used to create Fig. 6d panel.