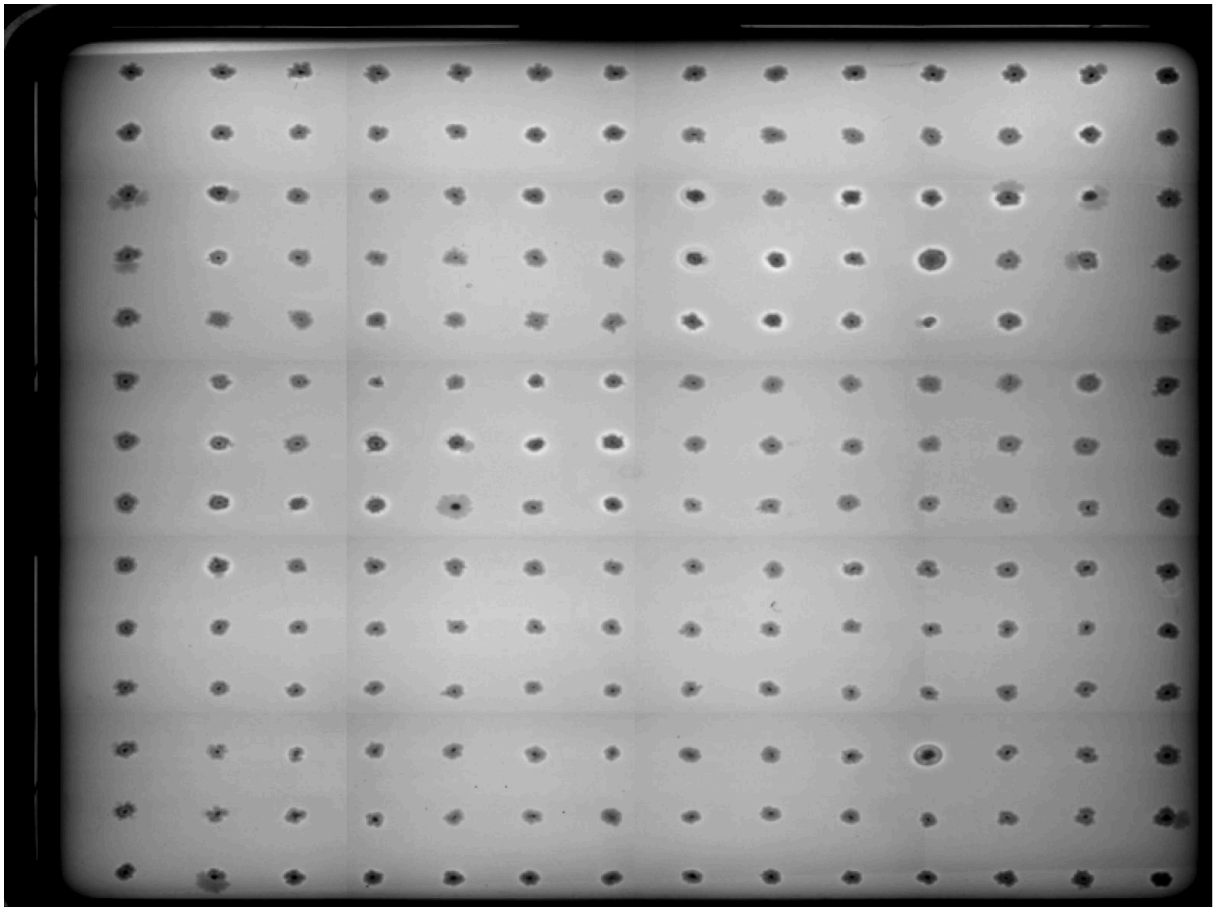
Supporting Information

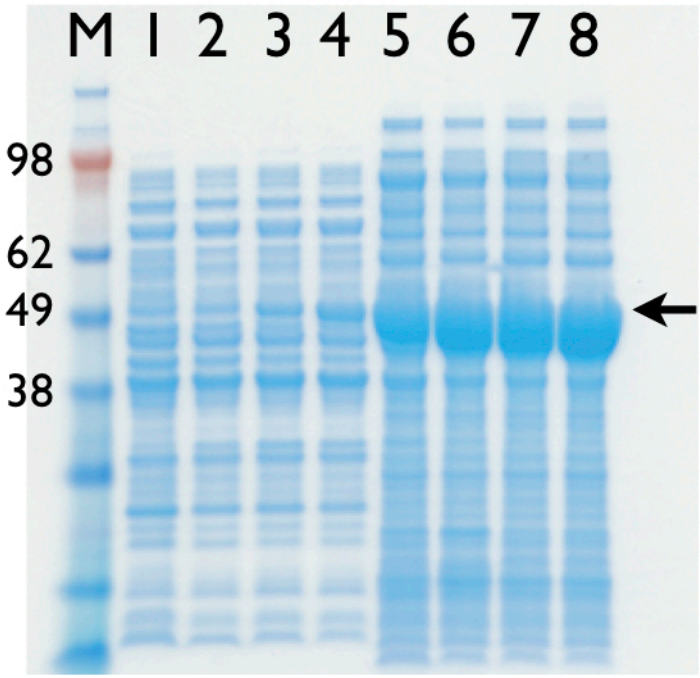
SI Figure 1

Plate clearing assay for active mutants. Colonies of *E. coli* expressing random mutants of TrzN were stabbed onto agar plates containing 0.9 g/L atrazine. When examined on a light box, translucent regions were visible around colonies with active protein.



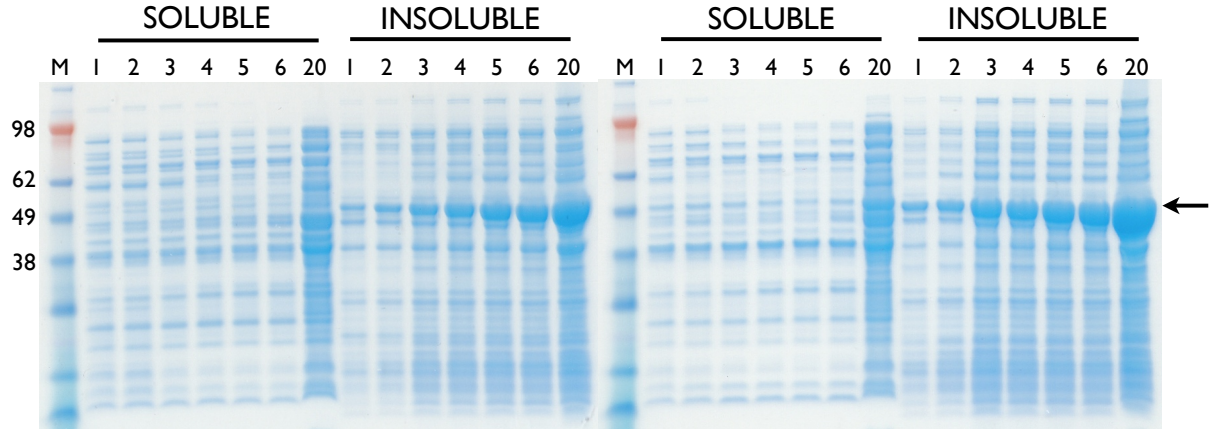
SI Figure 2

The effects of mutations on soluble (1-4) and insoluble (5-8) TrzN expression. Wild-type TrzN and mutants were expressed at 28 °C in TB medium with 1 μM IPTG for 24 hours. The level of both soluble and insoluble expression increases from TrzNWT (1, 5) to TrzNG1 (A159V; 2, 6), TrzNG2 (A159V/L131P; 3, 7) and TrzNG3 (A159V/L131P/D38N; 4, 8).



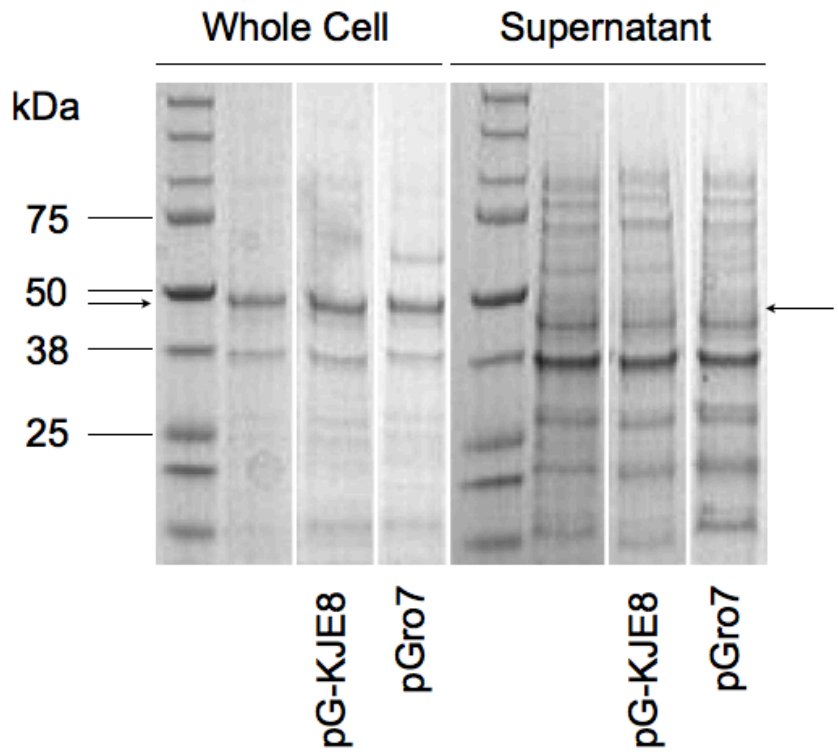
SI Figure 3

Reducing soluble expression of TrzNWt over time. Wild-type TrzN (left) and TrzNG3 (right) were induced with 1M IPTG and samples were taken at 4, 6, 8 and 24 hours. Insoluble and soluble fractions were analysed by SDS-PAGE. The relative expression of TrzNWT decreases over time, whereas TrzNG3 accumulates.



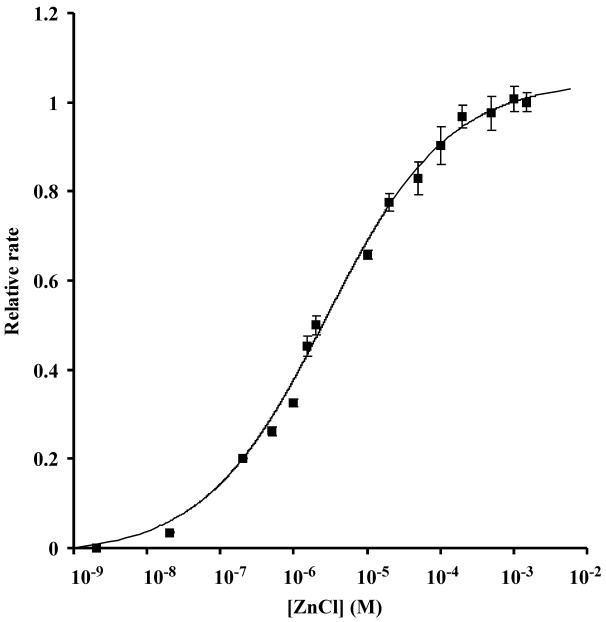
SI Figure 4

The effects of chaperones on TrzN expression in *E. coli.* Wild-type TrzN was co-expressed with and without various chaperones (pG-KJE8 = groES, groEL, dnak, dnaJ, grpE; pGro7 = groES, groEL). There is no increase in soluble expression as a result of chaperone overexpression.



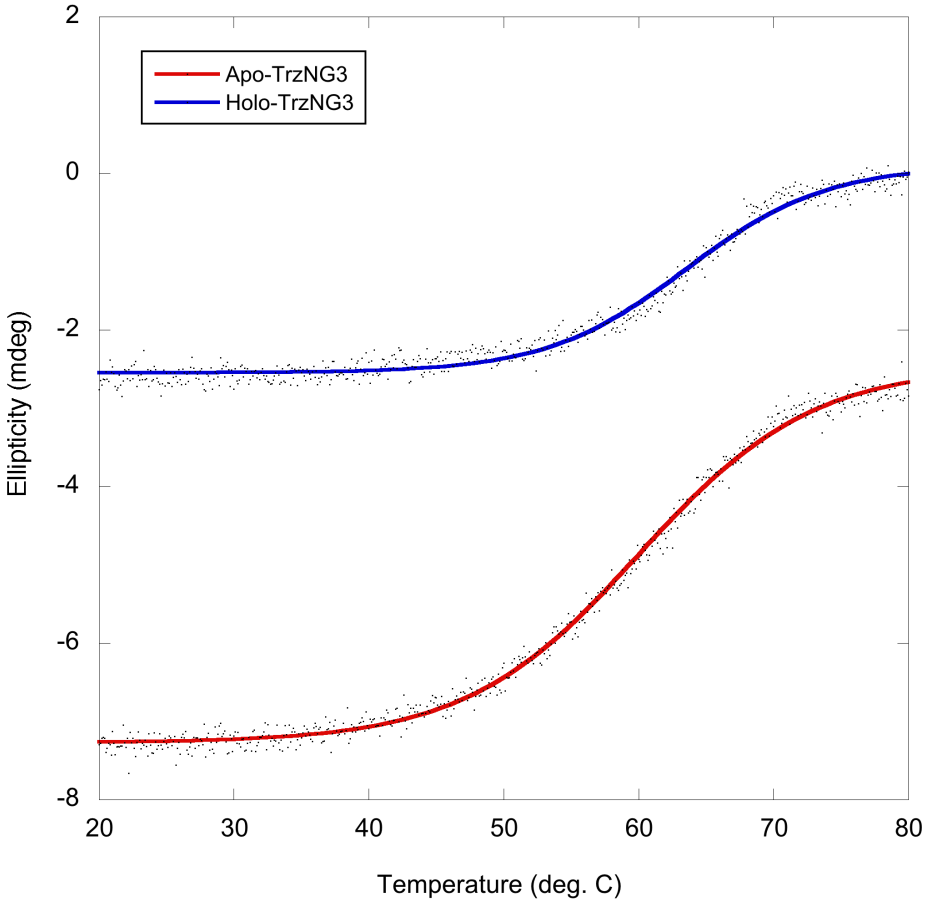
SI Fig 5

Metal saturation of TrzN. Apo-TrzNG3 was incubated with varying amounts of Zn^{2+} in storage buffer, before atrazine dechlorinase activity was assayed. The mid-point of the curve was calculated to be 49 μM.



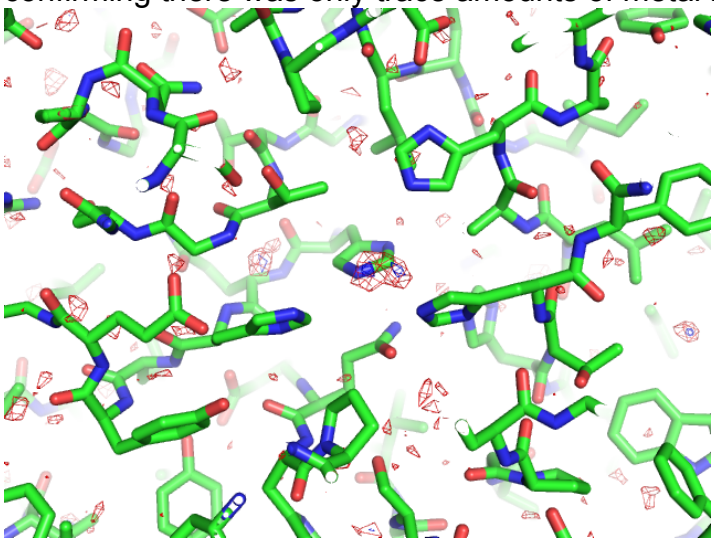
SI Fig 6

Circular dichroism spectroscopy of Apo-TrzNG3 and Holo-TrzNG3. The samples were heated from 20 °C to 80 °C at a rate of 1 °C per minute. Readings were taken at 220 nm every 0.1 °C increase in temperature. The temperature at which apo-TrzNG3 was 50% unfolded was 60.03 °C ± 0.1 °C (R-value = 0.997). The temperature at which holo-TrzNG3 was 50% unfolded was 63.53 °C ± 0.16 °C (R-value = 0.991).



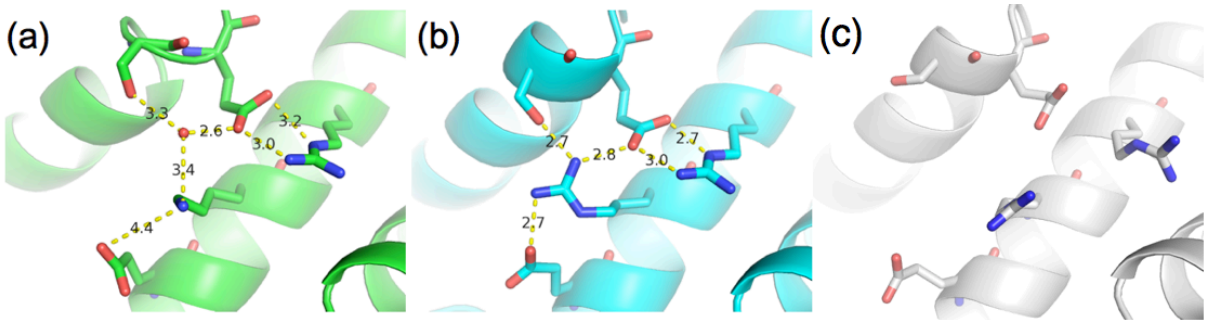
SI Fig 7

Anomalous Map of Apo-TrzNG3. The active site of subunit B and surrounding area is shown, overlaid with an anomalous difference map calculated from data collected above the Zn-K edge (1.2398 Å, *f*" = 3.635 e^-) to maximise anomalous scattering. As can be seen from the map, contoured at 2.5 σ and 4 σ (no density was present at 5 σ), there is no density at the metal ion binding site significantly above background, confirming there was only trace amounts of metal in the crystal structure.



SI Fig 8

Stabilization of Apo-PTE by the K185R mutation. The structure of Apo-PTE is shown (1PTA; a)[39], demonstrating that K185 on the surface of the protein interacts only loosely with its surrounding residues and a water molecule. In comparison, the K185R mutation (43ET; b)[40] results in two new salt bridges being formed and the surface water being displaced, consistent with a large stabilizing effect. However this is not correctly predicted by FoldX (c), where the new salt bridges are not accurately modelled.



SI Table 1. Oligonucleotides.

Primer	Function	Sequence (5'-3')
TrzNF	Amplification of codon optimised TrzN	AACCAACCACATATGATTCTGATCCGTGGTCTG
TrzNR	Amplification of codon optimised TrzN	GTTGGATCCTATCATTACAGATTTTTCGGAATCAGGGCCG
N38DF	D38N reversion	GTCGCTGTGGGTAAAGATTTAAGCGATCGTAGC
N38DR	D38N reversion	GCTACGATCGCTTAAATCTTTACCCACAGCGAC
P131LF	L131P reversion	GTCGCCGATCAGCATCTGTTTTTTCCAGGTGCA
P131LR	L131P reversion	TGCACCTGGAAAAAACAGATGCTGATCGGCGAC
V159AF	A159V reversion	ATTCGCTTCCATGCCGCGCGCAGCAGCATGACTCTG
V159AR	A159V reversion	CAGAGTCATGCTGCTGCGCGCGGCATGGAAGCGAAT

SI Table 2. Computational estimation of the effect of the A159V mutation on TrzN stability

State	TrzN-MD ΔΔG (kcal/mol)
apo	-4.29 (0.64)
holo	-0.43 (1.08)

Stability free energies (kcal/mol) for the reference state (a model of the unfolded state), apo protein and the Zn bound complex. Column 1 contains the MD-simulated free energies for changing alanine 159 into valine in apo-TrzN and holo-TrzN. The stability free energy difference of the A159V mutation can be written as $\Delta\Delta G = \Delta G_{prot} - \Delta G_{ref}$. A negative ΔΔG means the mutant is more stable. Error bars from the MDFE simulations are in parentheses.

SI Table 3. Computational estimation of the effects of mutations on apo-PTE and holo-PTE using FoldX

	holo-PTE	apo-PTE
K77E	**-1.46**	**-1.16**
A80V	-0.60	**-1.44**
R111S	0.04	0.14
K185R	-0.06	0.72
A204G	1.14	-0.33
D208G	1.07	0.23
I274S	0.73	0.21
R319S	0.68	**-1.52**

Stability free energies (ΔΔG, kcal/mol) for mutants of apo-PTE (1PTA) and holo-PTE (1HZY) as calculated using the protein design tool FoldX. A negative ΔΔG means the mutant is more stable.