Supporting Information

Detecting protein-protein interactions by Xe-129 NMR

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Experimental procedures

Plasmid preparation. Codon-optimized versions of the TEM-1 β-lactamase (TEM-1) enzyme fragments reported by Wehrman et al.[1] were synthesized by GenScript and cloned into a pRSFDuet-1 expression vector (Novagen) under the control of a T7 promoter. The first fragment, T1Fα, consisted of TEM-1 residues 26 through 197, followed by a stabilizing Asn-Gly-Arg tripeptide, $(GGGGS)_3$ linker repeat, then a cFos helix sequence (UniProt acc. no. P01100). The second fragment, T1Fω, consisted of TEM-1 residues 198 through 290, followed by a $(GGGGS)_3$ linker and then a cJun helix sequence (UniProt acc. no. P05412). The sequences for the cFos/cJun leucine zipper domain were referenced from Ransone et al.[2]

For the cFos deletion construct, a stop codon was inserted into the T1Fα gene between the $(GGGS)_3$ and c-Fos sequences via site-directed mutagenesis using the forward and reverse primers listed in Table S1. The mutated plasmid was amplified in NEB-5α competent *E. coli* cells (New England Biolabs) and then purified using a Miniprep Kit (Qiagen). The mutated T1Fα gene was sequenced at University of Pennsylvania DNA Sequencing Facility to verify the incorporation of the desired mutation and the integrity of the remaining gene sequence.

For protein purification purposes, the insert sequence of both fragments was obtained using BamHI (New England Biolabs) and AvrII (New England Biolabs) double digestion and cloned into a pCDFDuet-1 vector containing a 6xHis tag at the N-terminus of T1Fα fragment using T4 DNA ligase (New England Biolabs). The ligated plasmid was amplified in NEB-5α competent *E. coli* cells (New England Biolabs) and then purified using a Miniprep Kit (Qiagen).

Protein expression and purification. The plasmid pCDFDuet-1-T1F or pCDFDuet-1-T1F-STOPcFos was transformed into BL21(DE3) competent *E. coli* cells (New England Biolabs). The

cells were grown in three 2-liter baffled flasks containing 1 L of LB Miller broth supplemented with 50 μg/mL streptomycin to a final OD_{600} of 0.6 - 0.8, at which point the cells were induced with 1 mM IPTG. The cells were incubated overnight at 18 °C, pelleted by centrifugation, and frozen at -80 °C. The cell pellets were resuspended in 20 mM sodium phosphate (pH 7.4), 10 % (v/v) glycerol with one protease inhibitor tablet and lysed by sonication, at which point 0.5 M NaCl was added. The lysate was clarified by centrifugation at 15,000 rpm and the supernatant was loaded onto a HisTrap Ni-NTA column (GE Life Sciences) pre-equilibrated with 20 mM phosphate (pH 7.4), 0.5 M NaCl, 20 mM imidazole, 10 % (v/v) glycerol. The cJun-T1Fω fragment was eluted with 20 mM phosphate (pH 7.4), 0.5 M NaCl, 8 M urea and refolded by buffer exchange into 20 mM phosphate (pH 7.4), 0.5 M NaCl using a 10-kDa Amicon centrifugal tube. The T1Fα-cFos or T1Fα fragment was refolded on-column by removing urea in a step-wise manner with 20 mM phosphate (pH 7.4), 0.5 M NaCl, and eluted with 20 mM phosphate (pH 7.4), 0.5 M NaCl, 500 mM imidazole. Each fragment was concentrated and loaded onto a HiLoad 16/600 Superdex 75 pg size-exclusion column (GE Life Sciences). Pure proteins were pooled and concentrated. Protein purity was confirmed by SDS-PAGE (Figure S1). Protein concentrations were determined from the absorbance at 280 nm using the extinction coefficient 10,095 M^{-1} cm^{-1} for T1Fα-cFos and T1Fα, and 17,990 M^{-1} cm^{-1} for cJun-T1Fω, as calculated by PROTPRAM.[3] Secondary structures of refolded proteins were confirmed by circular dichroism (CD) spectroscopy (Figure S2).

Hyper-CEST NMR with purified TEM-1 fragments. Hyper-CEST z-spectra were acquired as described previously.[4] Hyperpolarized (hp) ^{129}Xe was generated using the spin-exchange optical pumping (SEOP) method with a home-built ^{129}Xe polarizer based on the IGI.Xe.2000 commercial model by GE. A gas mixture of 88% helium, 10% nitrogen, and 2% natural abundance xenon (Linde Group, NJ) was used as the hyperpolarizer input. ^{129}Xe hyperpolarization level was roughly

10-15%. For each data point in a hyper-CEST z-spectrum, hp ^{129}Xe was bubbled into the 10-mm NMR tube through capillaries for 20 s, followed by a 3-s delay to allow bubbles to collapse. Pressure of the gas downstream of the inlet valve to the NMR tube was ca. 63 psi and flow rate of gas was ca. 0.70 standard liters per minute. A d-SNOB saturation pulse with 690 Hz bandwidth was used.[5] Pulse length, τ_{pulse} = 3.80 ms; field strength $B_{1,max}$ = 77 μT; number of pulses, n_{pulse} = 600; saturation time, t_{sat} = 2.29 s. NMR experiments were performed using a Bruker 500 MHz NMR spectrometer and 10-mm PABBO probe at 300 K. A 90° hard pulse of this probe has a pulse length of 40.6 μs. Protein concentration used was 80 μM, with 0.1% (v/v) Pluronic L81 (Aldrich) added to mitigate foaming. In the time-dependent saturation transfer experiments, pulse length, τ_{pulse} = 1.0496 ms; field strength, $B_{1,max}$ = 279 μT. Both on-resonance and off-resonance data were fitted with first-order exponential decay curves. Saturation contrast was calculated as described before.[4] No significant protein aggregation was observed before and after all hyper-CEST experiments, as confirmed by dynamic light scattering (DLS, Figure S3).

Hyper-CEST NMR with TEM-1 fragments in *E. coli*. The plasmid pRSFDuet-1-T1F was transformed into BL21(DE3) *E. coli* competent cells. The cells were cultured on a LB-agar plate supplemented with 50 μg/mL kanamycin. A single colony of transformed cells was used to inoculate 5 mL of LB supplemented with 50 μg/mL kanamycin. The 5 mL culture was incubated overnight at 37 °C with shaking, and was then used to inoculate two 2-liter baffled flasks containing 1 L of LB medium supplemented with 50 μg/mL kanamycin. The 1 L cell cultures were incubated at 37 °C with shaking until OD_{600} reached 0.6-0.8. One flask was set aside and stored at 4 °C, while protein expression in the other flask was induced by 0.1 mM IPTG. The induced culture was incubated overnight at 18 °C with shaking. The next morning cells from both flasks were

pelleted, washed with cold PBS, and diluted in cold PBS to a final OD_{600} of 1. The same procedure was used for the cFos deletion construct.

Saturation transfer experiments with *E. coli* were performed as previously described.[4] Briefly, hp ^{129}Xe was bubbled into the 10-mm NMR tube through capillaries for 20 s, followed by a 3-s delay to allow bubbles to collapse. Pressure of the gas downstream of the inlet valve to the NMR tube was ca. 63 psi and flow rate of gas was ca. 0.70 standard liters per minute. Saturation frequencies of d-SNOB pulses were positioned at +60 ppm and -60 ppm, referenced to the ^{129}Xe@aq peak for on- and off-resonance, respectively. Pulse length, τ_{pulse} = 1.0496 ms; field strength, $B_{1,max}$ = 279 μT. Both on-resonance and off-resonance data were fitted with first-order exponential decay curves. According to a previous study using the same procedures and *E. coli* strain, we estimate that ~80% cells were viable during hyper-CEST experiments.[6]

Table S1. Oligonucleotide primers used in T1Fα site-directed mutagenesis to generate cFos deletion construct.

Forward primer	5′ – GGCAGCGGTGGCGGTGGCAGCTAAGATACCCTGCAGGCGGAAACC – 3′
Reverse primer	5′ – GGTTTCCGCCTGCAGGGTATCTTAGCTGCCACCGCCACCGCTGCC – 3′

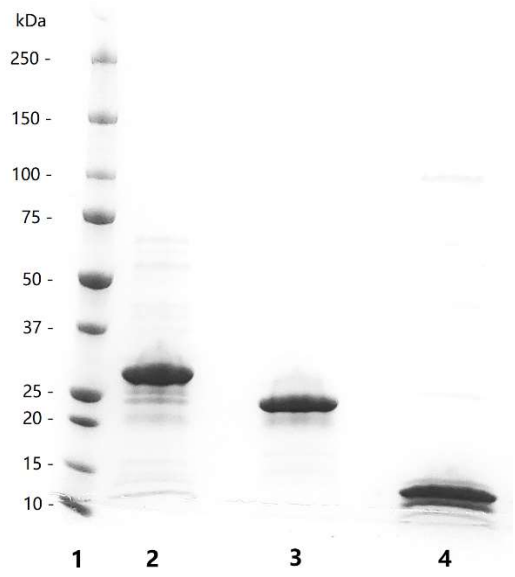

Figure S1. SDS-PAGE analysis of purified TEM-1 fragments. Lane 1: Precision plus protein standards (Bio-Rad); lane 2: T1Fα-cFos (25.9 kDa); lane 3: T1Fα (22.4 kDa); lane 4: cJun-T1Fω (14.6 kDa).

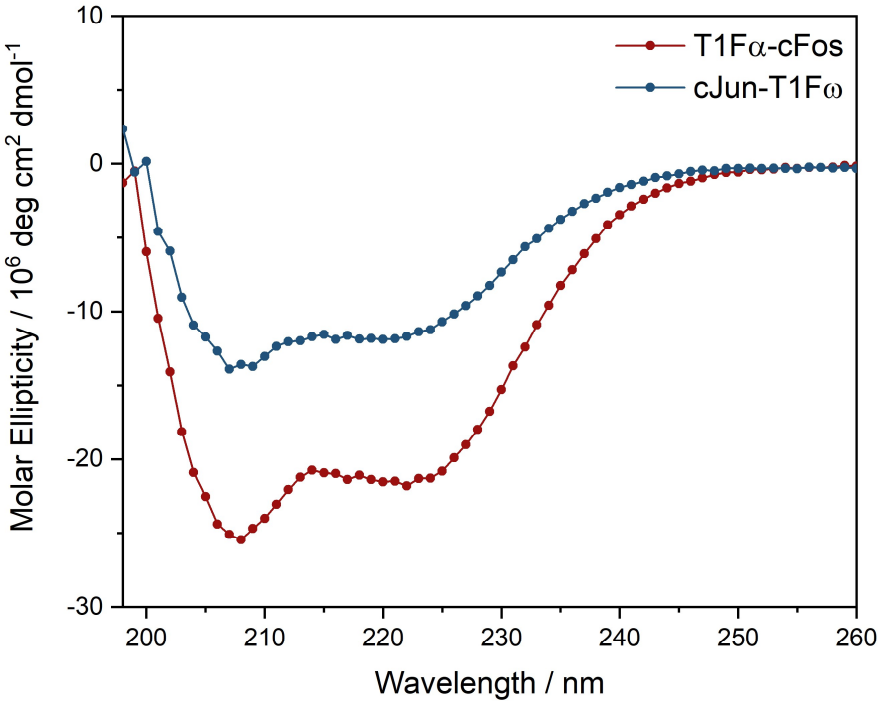

Figure S2. CD spectra of refolded TEM-1 fragments in 50 mM Tris (pH 7.4), 500 mM NaCl at 298 K.

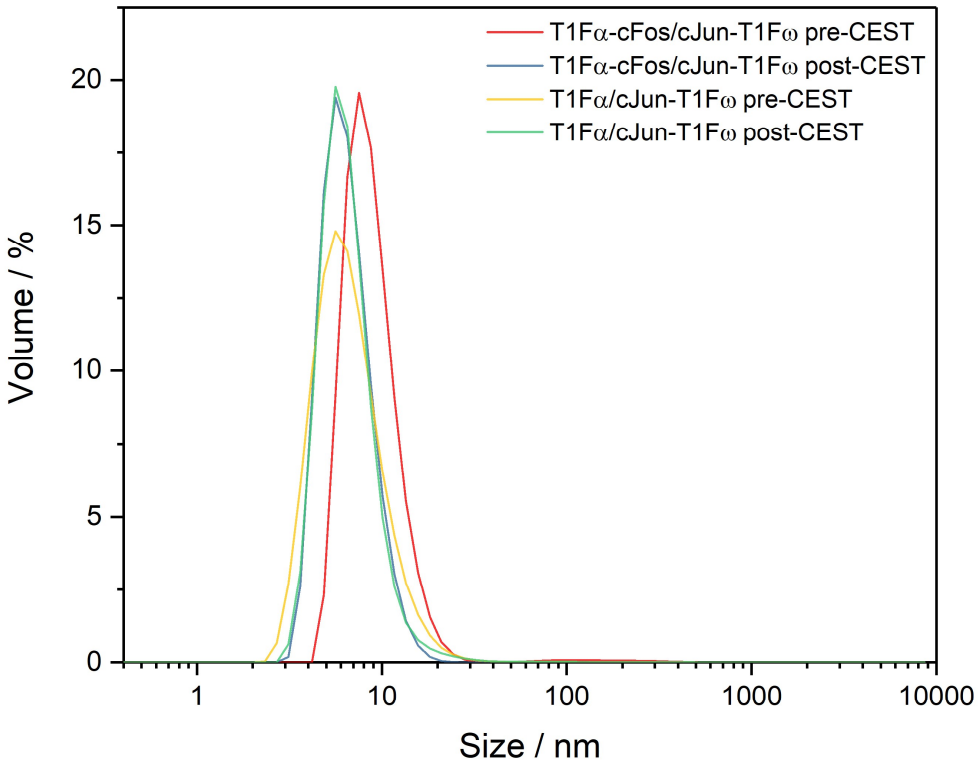

Figure S3. DLS data of size distribution of TEM-1 fragments by volume in 50 mM Tris (pH 7.4), 500 mM NaCl at 298 K. Red: 80 μM T1Fα-cFos/cJun-T1Fω pre-CEST sample, average diameter = 9.0 ± 0.6 nm; blue: 80 μM T1Fα-cFos/cJun-T1Fω post-CEST sample, average diameter = 6.7 ± 0.2 nm; yellow: 80 μM T1Fα/cJun-T1Fω pre-CEST sample, average diameter = 7.1 ± 0.6 nm; 80 μM T1Fα/cJun-T1Fω post-CEST sample, average diameter = 6.9 ± 0.7 nm.

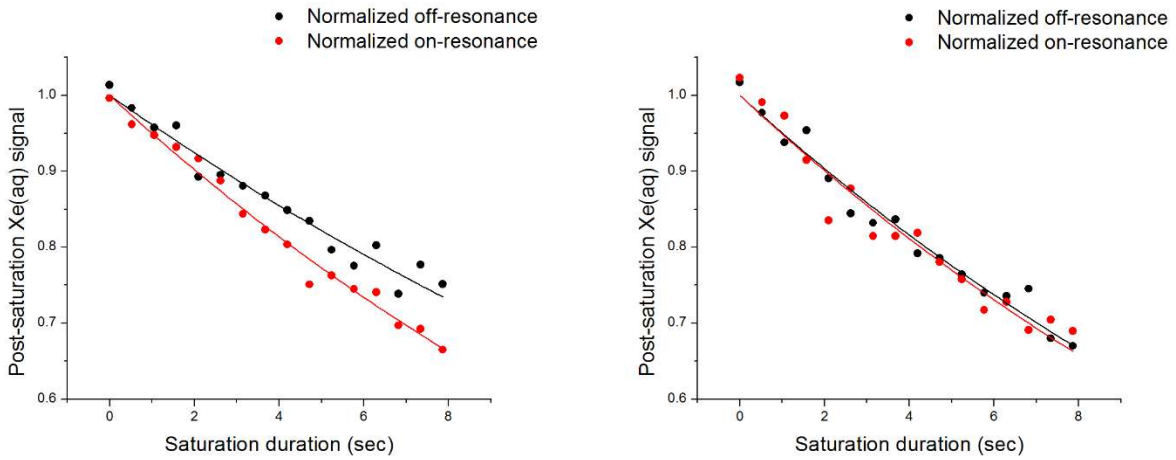

Figure S4. Time-dependent saturation transfer data for 1 μM TEM-1 fragments in 50 mM Tris (pH 7.4), 500 mM NaCl at 300 K. Left: T1Fα-cFos/cJun-T1Fω; T_{1on} = 19.4 ± 0.6 s; T_{1off} = 25.5 ± 1.4 s; saturation contrast = 0.06 ± 0.01. Right: T1Fα/cJun-T1Fω; T_{1on} = 19.1 ± 1.2 s; T_{1off} = 19.7 ± 0.9 s; saturation contrast = 0.01 ± 0.01. Each measurement was repeated three times.

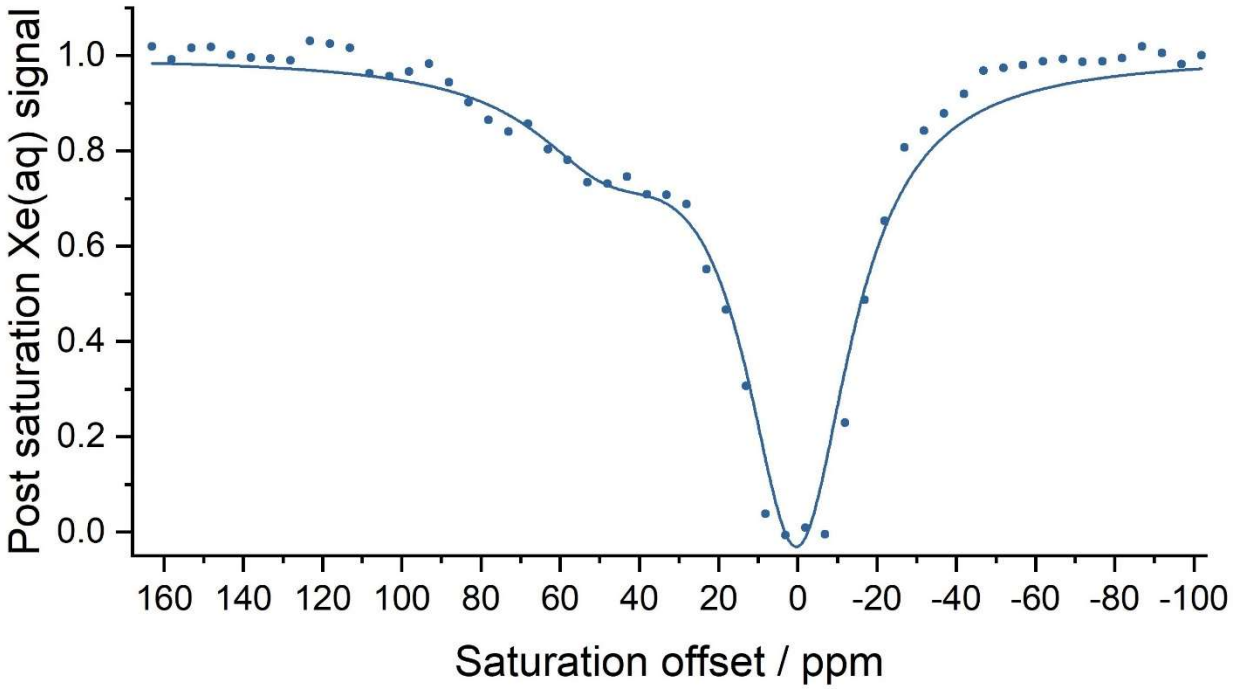

Figure S5. Z-spectrum of 80 μM T1Fα/cJun-T1Fω in 50 mM Tris (pH 7.4), 500 mM NaCl at 300 K. Saturation time, t_{sat} = 2.29 s; field strength, $B_{1,max}$ = 77 μT. The solid circles show the experimental data, and the line shows the Lorentzian fit. Each data point is the average of three measurements.

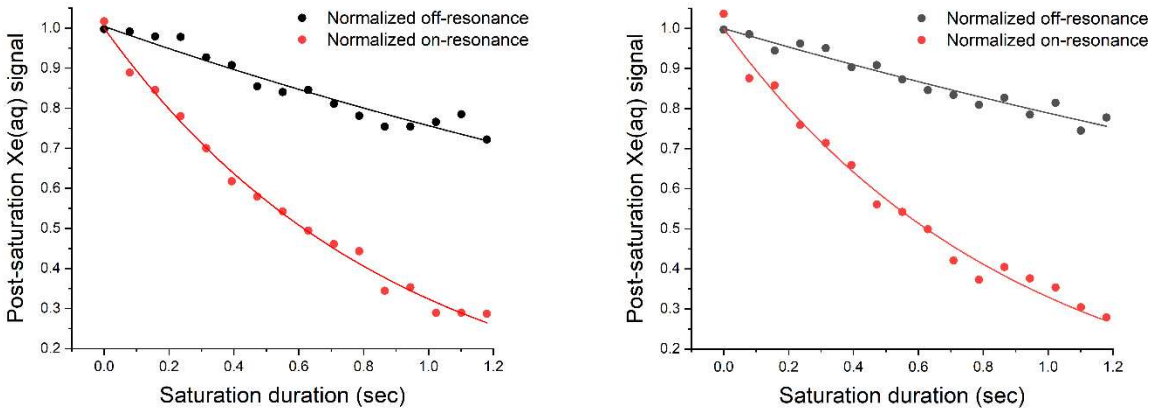

Figure S6. Time-dependent saturation transfer data for 80 μM TEM-1 fragments in 50 mM Tris (pH 7.4), 500 mM NaCl at 300 K. Left: T1Fα-cFos/cJun-T1Fω; T_{1on} = 0.89 ± 0.02 s; T_{1off} = 3.53 ± 0.28 s; saturation contrast = 0.42 ± 0.02. Right: T1Fα/cJun-T1Fω; T_{1on} = 0.90 ± 0.02 s; T_{1off} = 4.22 ± 0.14 s; saturation contrast = 0.44 ± 0.01. Each measurement was repeated three times.

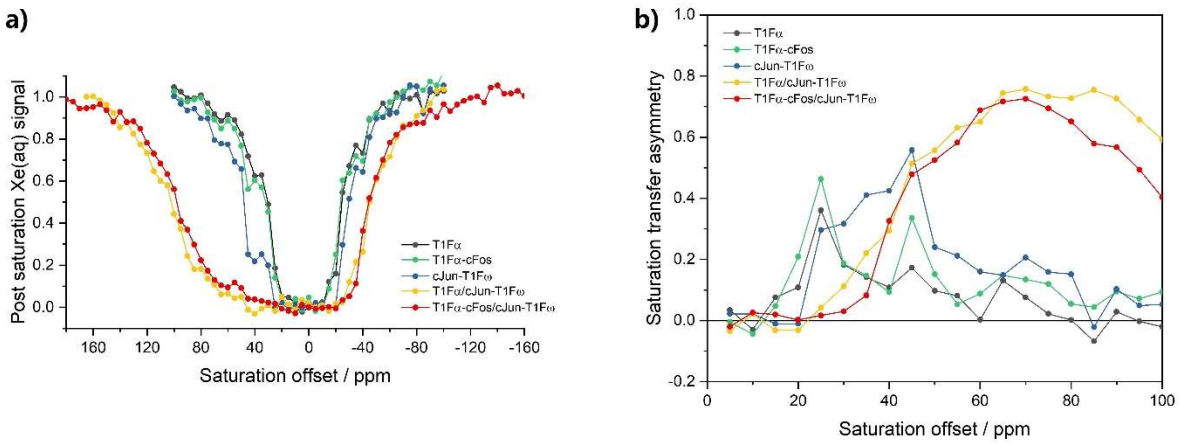

Figure S7. Hyper-CEST data for TEM-1 fragments using high pulse power. a) Z-spectrum of 80 μM T1Fα, T1Fα-cFos, cJun-T1Fω, T1Fα/cJun-T1Fω and T1Fα-cFos/cJun-T1Fω in 50 mM Tris (pH 7.4), 500 mM NaCl at 300 K. Saturation time, t_{sat} = 2.29 s; field strength, $B_{1,max}$ = 279 μT. b) Asymmetry in saturation transfer around the ^{129}Xe@aq signal. Saturation transfer asymmetry is defined as the difference between the normalized post-saturation ^{129}Xe signals at the negative offsets and the corresponding positive offsets. Note that ^{129}Xe signals of T1Fα/cJun-T1Fω and T1Fα-cFos/cJun-T1Fω samples were almost fully saturated up to +60 ppm due to the broad ^{129}Xe@aq peak, and the ^{129}Xe@T1Fα/cJun-T1Fω and ^{129}Xe@T1Fα-cFos/cJun-T1Fω signals extended to negative offsets, which resulted in relatively smaller saturation transfer asymmetry values from 0 to 60 ppm.

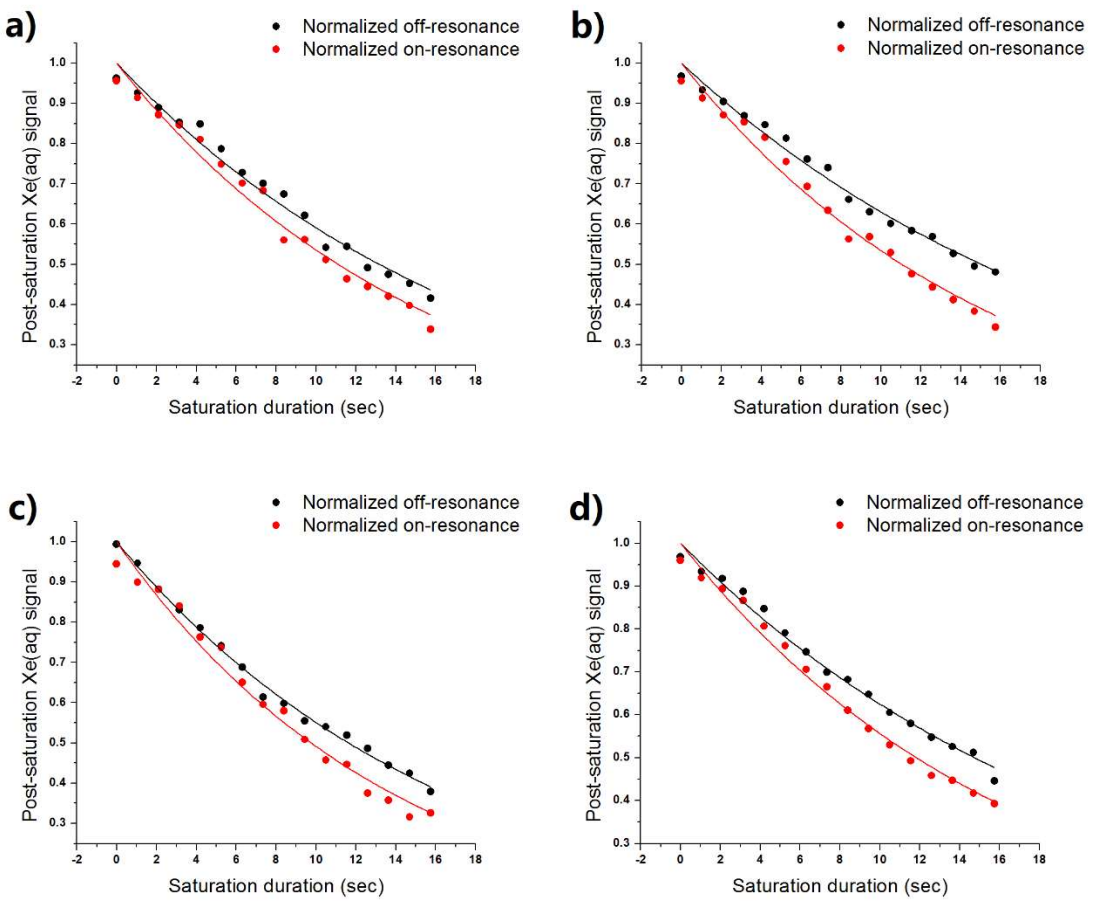

Figure S8. Time-dependent saturation decay curves for *E. coli* samples expressing TEM-1 fragments, in PBS at 300 K. a) T1Fα-cFos/cJun-T1Fω, non-induced; T_{1on} = 16.0 ± 0.6 s; T_{1off} = 19.0 ± 0.7 s; saturation contrast = 0.10 ± 0.02. b) T1Fα-cFos/cJun-T1Fω, induced by IPTG; T_{1on} = 16.0 ± 0.6 s; T_{1off} = 21.7 ± 0.7 s; saturation contrast = 0.18 ± 0.02. c) T1Fα/cJun-T1Fω, non-induced; T_{1on} = 14.1 ± 0.6 s; T_{1off} = 16.8 ± 0.3 s; saturation contrast = 0.12 ± 0.02. d) T1Fα/cJun-T1Fω, induced by IPTG; T_{1on} = 17.1 ± 0.5 s; T_{1off} = 21.3 ± 0.6 s; saturation contrast = 0.12 ± 0.02. Each measurement was repeated three times.

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