Supplementary Information

Plasticity, dynamics, and inhibition of emerging tetracycline-resistance enzymes

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This file includes Supplementary Results (Supplementary Figures/Legends 1-10, Supplementary Tables 1-5)

Supplementary Results

Supplementary Figure 1 | Overall structures and FAD conformation states of Tet(50,51,55,56)

Crystal structures of **a,** Tet(50) monomer A, **b,** Tet(50) monomer B, **c,** Tet(51), **d,** Tet(55), and **e,** Tet(56).

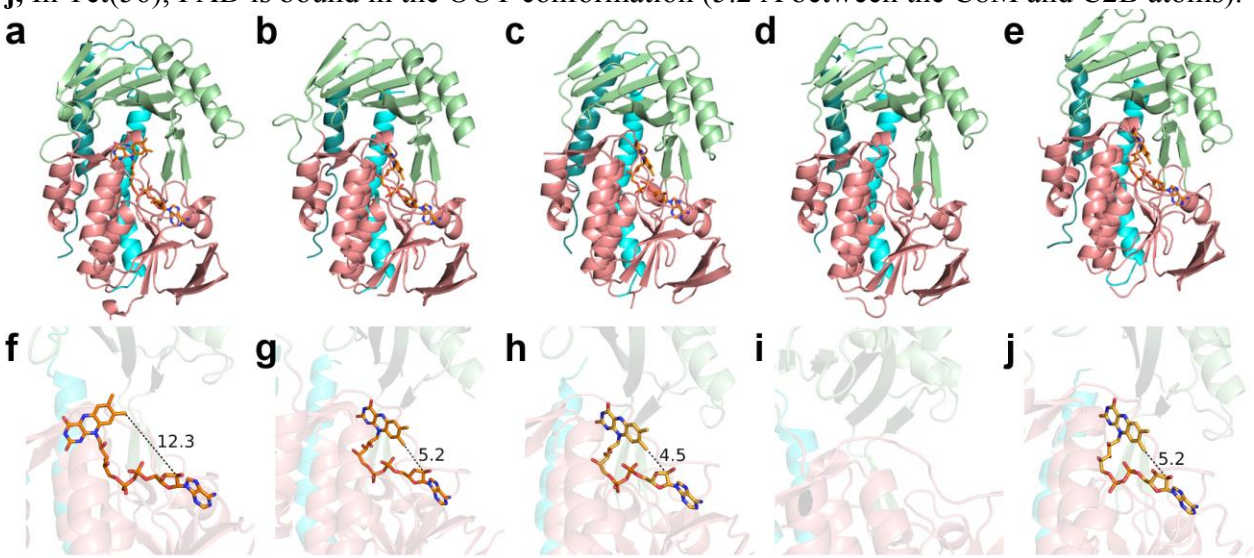
f, In Tet(50) monomer A, FAD is bound non-covalently in the IN conformation, characterized by a 12.3 Å distance between the C8M and C2B atoms of the FAD molecule.

g, In Tet(50) monomer B, FAD is bound in the OUT conformation (5.2 Å between the C8M and C2B atoms).

h, In Tet(51), FAD is bound in the OUT conformation (4.5 Å between the C8M and C2B atoms).

i, In Tet(55), no electron density for ordered FAD is observed.

j, In Tet(56), FAD is bound in the OUT conformation (5.2 Å between the C8M and C2B atoms).



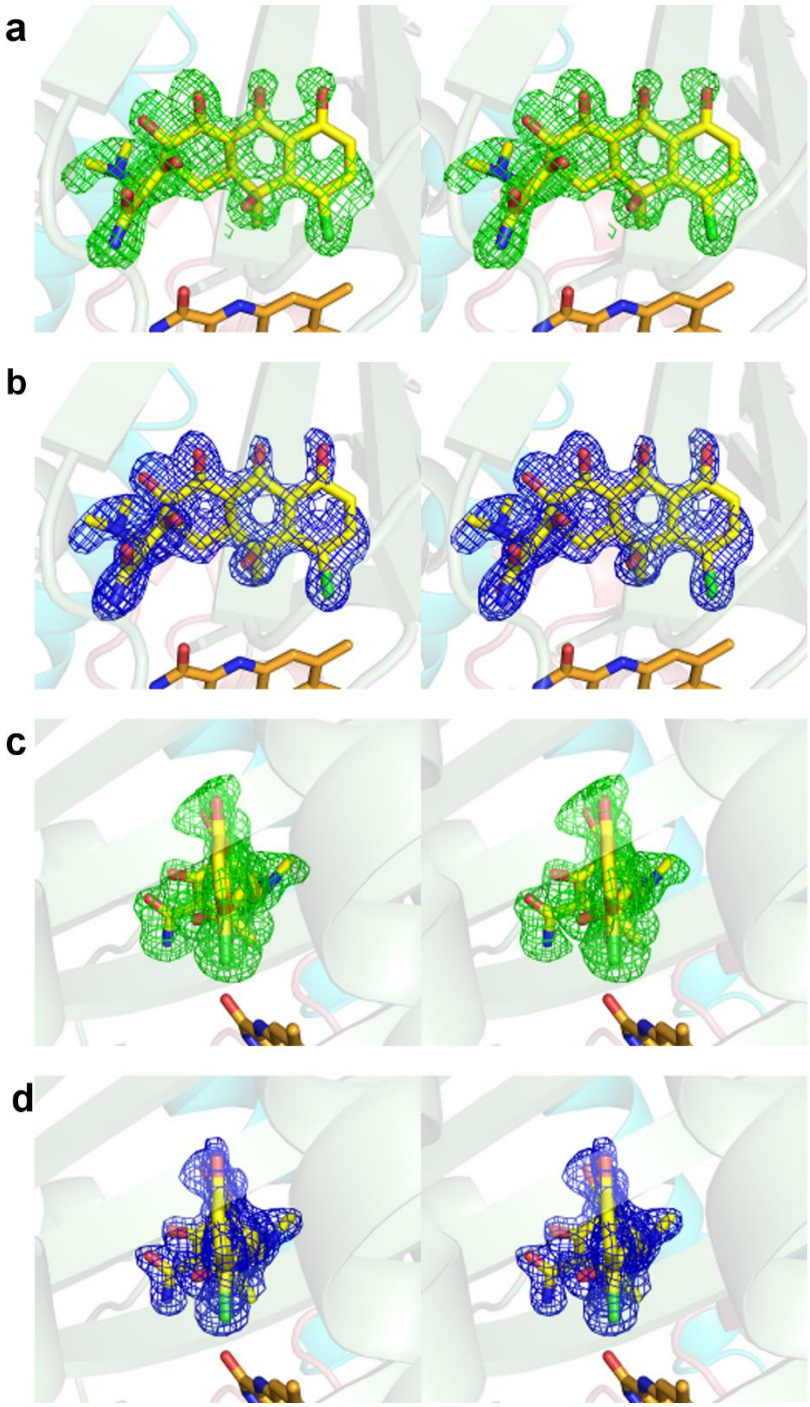
Supplementary Figure 2 | Chlortetracycline has a distinctive three-dimensional architecture with a significant bend between rings A and B, allowing for unambiguous modeling into the electron density

a, The F_o - F_c map (contoured at 2.0σ) before modeling of chlortetracycline.

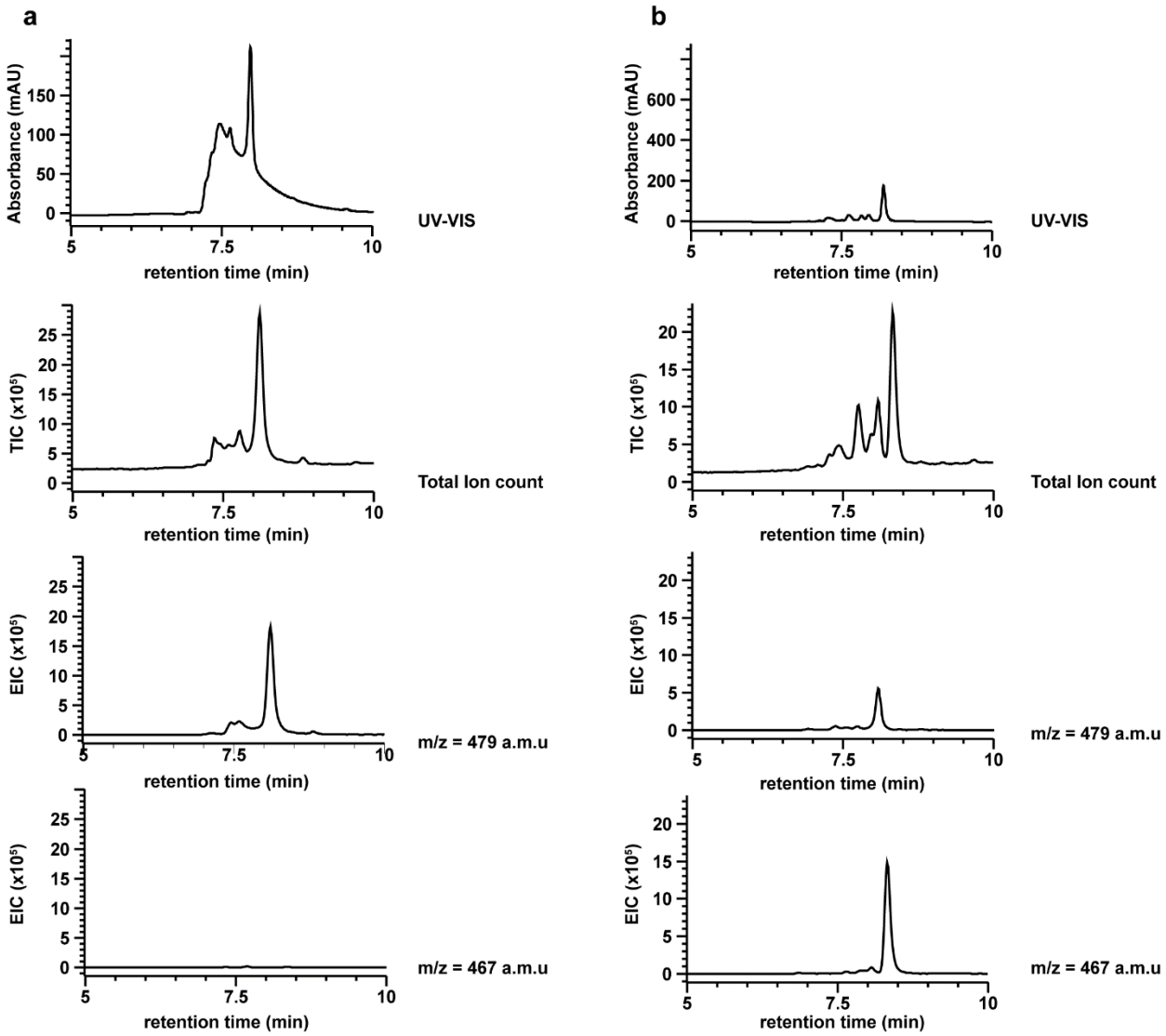
b, The $2F_o$ - F_c map (contoured at 1.0σ) after modeling of chlortetracycline.

c, Rotated view of the F_o - F_c map (contoured at 2.0σ) before modeling of chlortetracycline.

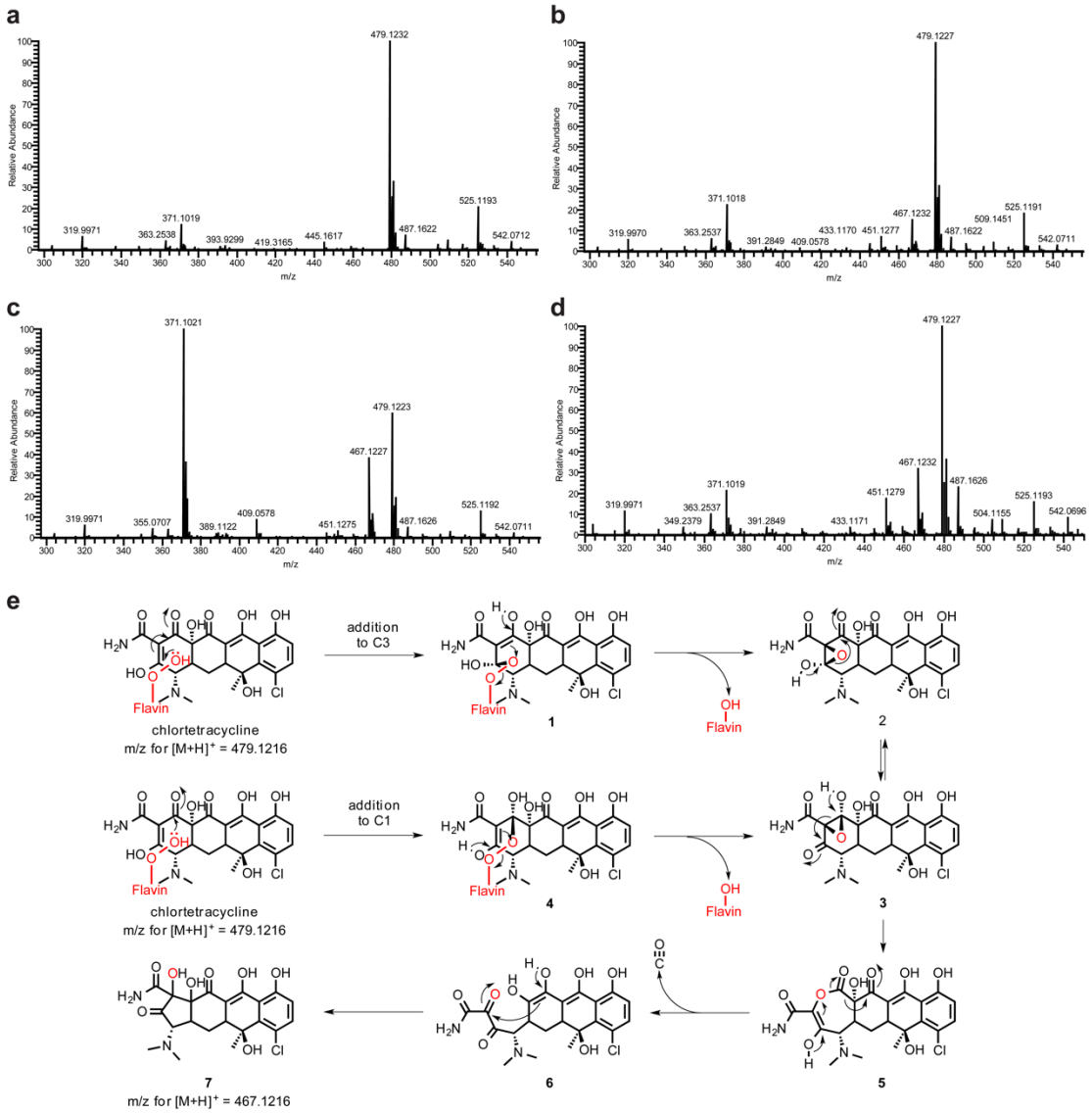
d, Rotated view of the $2F_o$ - F_c map (contoured at 1.0σ) after modeling of chlortetracycline.



Supplementary Figure 3 | Low-resolution LC-MS analysis of tetracycline destructase reaction with chlortetracycline shows clean conversion to the *m/z* 467 oxidation product. (a) LC-MS chromatograms taken after 10 minutes of the chlortetracycline no-enzyme control reaction. UV-Vis chromatograms show absorbance at 260 nm. The TIC and EIC chromatograms show that only chlortetracycline (*m/z* for $[M+H]^+$ = 479; retention time = 8.1 mins) is present in the reaction mixture. (**b**) LC-MS chromatograms taken after 10 min of the chlortetracycline reaction with Tet(55). The TIC and EIC chromatograms show that the majority of the chlortetracycline (*m/z* for $[M+H]^+$ = 479; retention time = 8.1 mins) was converted to the oxidation product (*m/z* for $[M+H]^+$ = 467; retention time = 8.3 mins). TIC = total ion chromatogram; EIC = extracted ion chromatogram.



Supplementary Figure 4 | High-resolution MS-MS analysis of enzymatic reactions with chlortetracycline supports conversion to the *m/z* 467.12 oxidation product. (**a**) MS spectrum of no enzyme control; HRMS (ESI) calculated for $C_{22}H_{24}ClN_2O_8^+$ (chlortetracycline): 479.1216 [$(M+H)^+$], observed 479.1232. (**b**) MS spectrum of chlortetracycline after reaction with Tet(50). (**c**) MS spectrum of chlortetracycline after reaction with Tet(55). (**d**) MS spectrum of chlortetracycline after reaction with Tet(56). **(f)** Two proposed mechanisms for degradation of chlortetracycline, consistent with MS and crystallographic data. Addition of the C4a flavin peroxide to C3 generates intermediate **1**, which can undergo epoxide formation to give an equilibrating mixture of intermediates **2** and **3**. Intermediate **3** can also be generated via intermediate **4** arising from direct attack of the C4a flavin peroxide on carbonyl C1. Intermediate **3** can rearrange to cycloheptanone intermediate **5**. Fragmentation will give intermediate **6** via loss of carbon monoxide followed by ring contraction resulting in formation of product **7** with *m/z* 467 for $[M+H]^+$.



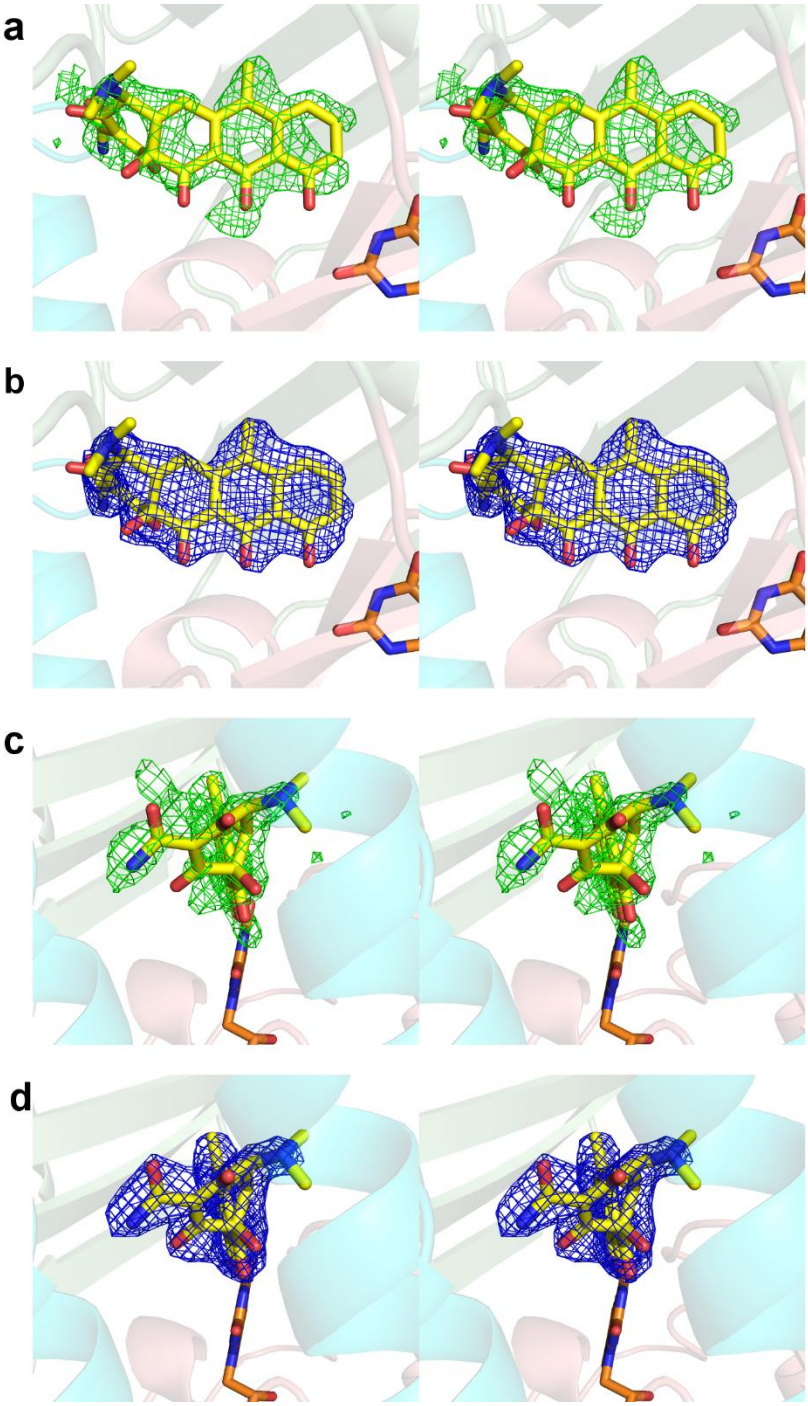
Supplementary Figure 5 | Anhydrotetracycline has a distinctive three-dimensional architecture with a significant bend between rings A and B, allowing for unambiguous modeling into the electron density

a, The F_o - F_c map (contoured at 2.0σ) before modeling of anhydrotetracycline.

b, The $2F_o$ - F_c map (contoured at 1.0σ) after modeling of anhydrotetracycline.

c, Rotated view of the F_o - F_c map (contoured at 2.0σ) before modeling of anhydrotetracycline.

d, Rotated view of the $2F_o$ - F_c map (contoured at 1.0σ) after modeling of anhydrotetracycline.

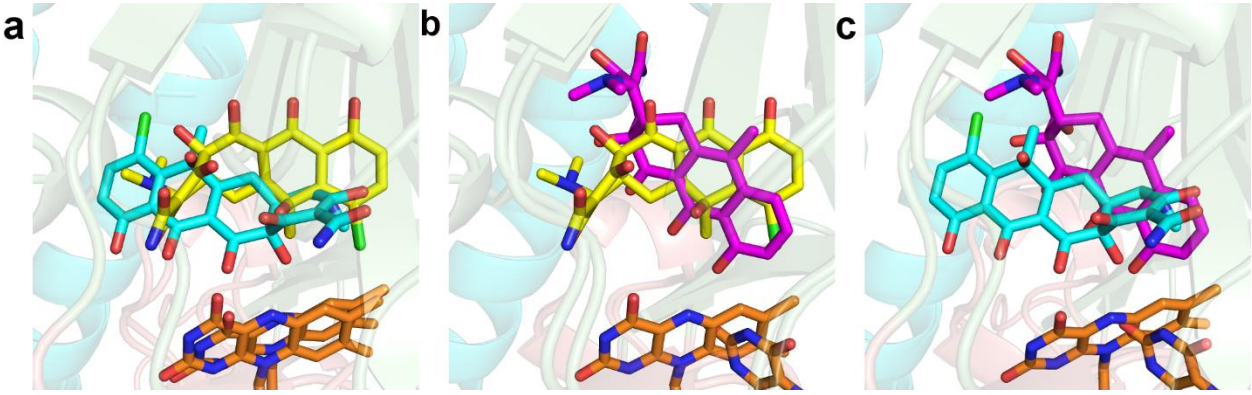


Supplementary Figure 6 | Superimposition of tetracycline compounds in substrate-bound structures

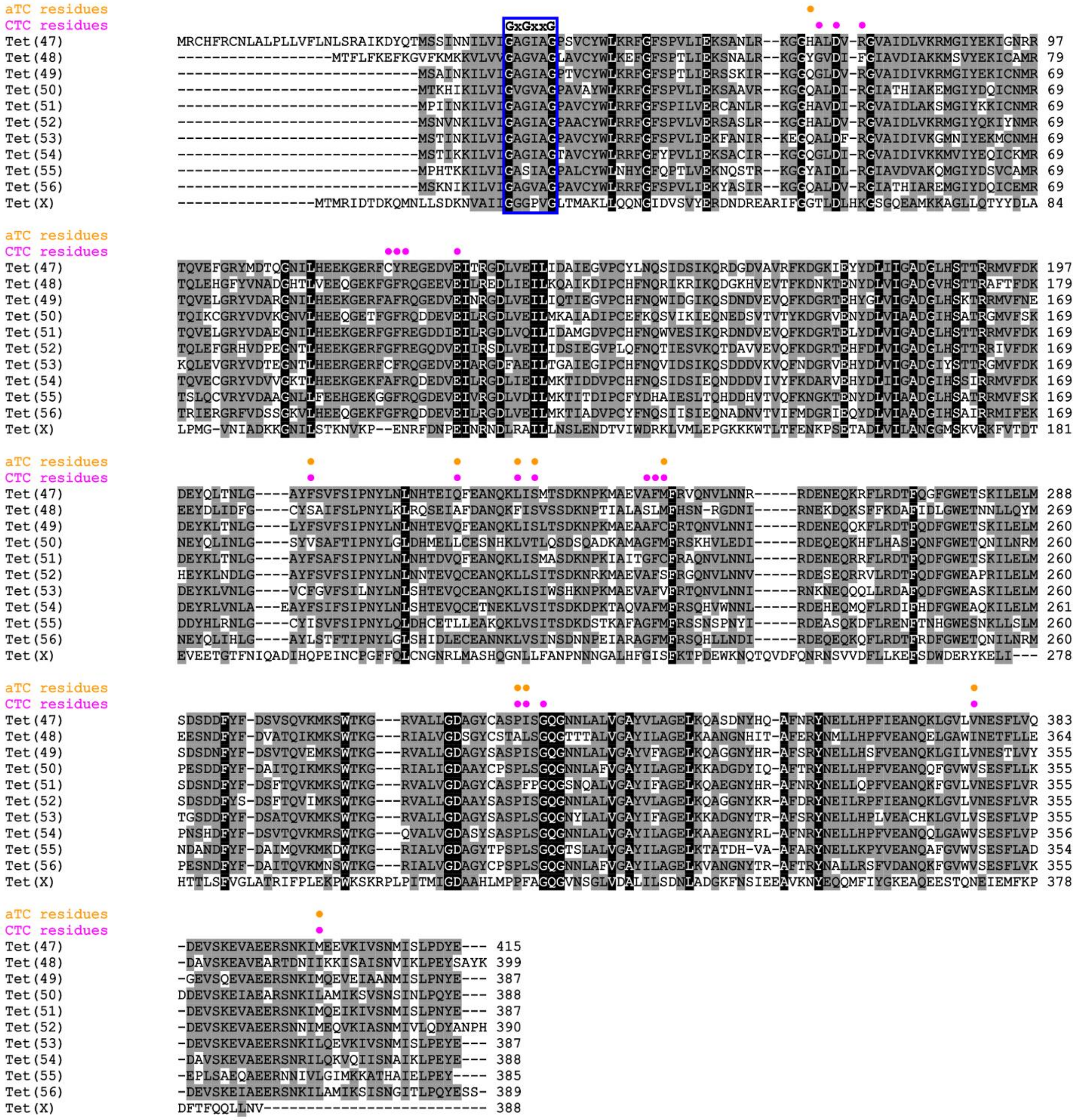
a, Superimposition of the Tet(50)+chlortetracycline (yellow) and Tet(X)+chlortetracycline (cyan) structures.

b, Superimposition of the Tet(50)+chlortetracycline (yellow) and Tet(50)+anhydrotetracycline (magenta) structures.

c, Superimposition of the Tet(X)+chlortetracycline (cyan) and Tet(50)+anhydrotetracycline (magenta) structures.



Supplementary Figure 7 | Multiple sequence alignment of Tet(47-56, X). Tetracycline destructases have high levels of sequence similarity in the residues important for binding of anhydrotetracycline (aTC, orange) or chlortetracycline (CTC, pink). Alignment includes Tet(X), which shares at most 24.4% amino acid identity with Tet(47-56). Conserved FAD binding motif is boxed in blue.



```
aTC residues                                                                             ●
CTC residues                                          GxGxxG                              ● ●  ●
Tet(47)         MRCHFRCNLALPLLVFLNLSRAIKDYQTMSSINNILVIGAGIAGPSVCYWLKRFGFSPVLIEKSANLR--KGGHALDV-RGVAIDLVKRMGIYEKIGNRR 97
Tet(48)         ------------------MTFLFKEFKGVFKMKKVLVVGAGVAGLAVCYWLKEFGFSPTLIEKSNALR--KGGYGVDI-FGIAVDIAKKMSVYEKICAMR 79
Tet(49)         ----------------------------MSAINKILVIGAGIAGPTVCYWLKRFGFSPTLIERSSKIR--KGGQGLDV-RGVAIDIVKRMGIYEKICNMR 69
Tet(50)         ----------------------------MTKHIKILVIGVGVAGPAVAYWLKRFGFSPVLIEKSAAVR--KGGQALDI-RGIATHIAKEMGIYDQICNMR 69
Tet(51)         ----------------------------MPIINKILVIGAGIAGPAVCYWLRRFGFSPILVERCANLR--KGGHAVDI-RGVAIDLAKSMGIYKKICNMR 69
Tet(52)         ----------------------------MSNVNKILVIGAGIAGPAACYWLRRFGFSPVLVERSASLR--KGGHALDV-RGVAIDLVKRMGIYQKIYNMR 69
Tet(53)         ----------------------------MSTINKILVIGAGIAGPAVCYWLRRFGFSPVLIEKFANIR--KEGQALDF-RGVAIDIVKGMNIYEKMCNMH 69
Tet(54)         ----------------------------MSTIKKILVIGAGIAGTAVCYWLRRFGFYPVLIEKSACIR--KGGQGLDI-RGVAIDIVKKMVIYEQICKMR 69
Tet(55)         ----------------------------MPHTKKILVIGASIAGPALCYWLNHYGFQPTLVEKNQSTR--KGGYAIDL-RGIAVDVAKQMGIYDSVCAMR 69
Tet(56)         ----------------------------MSKNIKILVIGAGVAGPAVCYWLRRFGFSPVLIEKYASIR--KGGQALDV-RGIATHIAREMGIYDQICEMR 69
Tet(X)          ----------------MTMRIDTDKQMNLLSDKNVAIIGGGPVGLTMAKLLQQNGIDVSVYERDNDREARIFGGTLDLHKGSGQEAMKKAGLLQTYYDLA 84

aTC residues
CTC residues                            ●●●     ●
Tet(47)         TQVEFGRYMDTQGNILHEEKGERFCYREGEDVEITRGDLVEILIDAIEGVPCYLNQSIDSIKQRDGDVAVRFKDGKIEYYDLIIGADGLHSTTRRMVFDK 197
Tet(48)         TQLEHGFYVNADGHTLVEEQGEKFGFRQGEEVEILREDLIEILKQAIKDIPCHFNQRIKRIKQDGKHVEVTFKDNKTENYDLVIGADGVHSTTRAFTFDK 179
Tet(49)         TQVELGRYVDARGNILHEEKGERFAFRQGEDVEINRGDLVEILIQTIEGVPCHFNQWIDGIKQSDNDVEVQFKDGRTEHYGLVIGADGLHSKTRRMVFNE 169
Tet(50)         TQIKCGRYVDVKGNVLHEEQGETFGFRQDDEVEILRGDLVEILMKAIADIPCEFKQSVIKIEQNEDSVTVTYKDGRVENYDLVIAADGIHSATRGMVFSK 169
Tet(51)         TQVELGRYVDAEGNILHEEKGERFGFREGDDIEILRGDLVQILIDAMGDVPCHFNQWVESIKQRDNDVEVQFKDGRTELYDLVIGADGLHSTTRRMVFDK 169
Tet(52)         TQLEFGRHVDPEGNTLHEEKGERFGFREGQDVEIIRSDLVEILIDSIEGVPLQFNQTIESVKQTDAVVEVQFKDGRTEHFDLVIGADGLHSTTRRIVFDK 169
Tet(53)         KQLEVGRYVDTEGNTLHEERGERFCFRQGEDVEIARGDFAEILTGAIEGIPCHFNQVIDSIKQSDDDVKVQFNDGRVEHYDLVIGADGIYSTTRGMVFDK 169
Tet(54)         TQVECGRYVDVVGKTLHEEKGEKFAFRQDEDVEILRGDLIEILMKTIDDVPCHFNQSIDSIEQNDDDVIVYFKDARVEHYDLIIGADGIHSSIRRMVFDK 169
Tet(55)         TSLQCVRYVDAAGNLLFEEHGEKGGFRQGDEVEIVRGDLVDILMKTITDIPCFYDHAIESLTQHDDHVTVQFKNGKTENYDLVIAADGLHSATRRMVFSK 169
Tet(56)         TRIERGRFVDSSGKVLHEEQGEKFGFRQDDEVEILRGDLVEILMKTIADVPCYFNQSIISIEQNADNVTVIFMDGRIEQYDLVIAADGIHSAIRRMIFEK 169
Tet(X)          LPMG-VNIADKKGNILSTKNVKP--ENRFDNPEINRNDLRAILLNSLENDTVIWDRKLVMLEPGKKKWTLTFENKPSETADLVILANGGMSKVRKFVTDT 181

aTC residues                   ●                ●      ● ●              ●
CTC residues                   ●                ●      ● ●            ●●●
Tet(47)         DEYQLTNLG----AYFSVFSIPNYLNLNHTEIQFEANQKLISMTSDKNPKMAEVAFMFRVQNVLNNR-----RDENEQKRFLRDTFQGFGWETSKILELM 288
Tet(48)         EEYDLIDFG----CYSAIFSLPNYLKLRQSEIAFDANQKFISVSSDKNPTIALASLMFHSN-RGDNI-----RNEKDQKSFFKDAFIDLGWETNNLLQYM 269
Tet(49)         DEYKLTNLG----LYFSVFSIPNYLNLNHTEVQFEANQKLISLTSDKNPKMAEAAFCFRTQNVLNNI-----RDENEQQKFLRDTFQDFGWETSKILELM 260
Tet(50)         NEYQLINLG----SYVSAFTIPNYLGLDHMELLCESNHKLVTLQSDSQADKAMAGFMFRSKHVLEDI-----RDEQEQKHFLHASFQNFGWETQNILNRM 260
Tet(51)         DEYKLTNLG----AYFSAFSIPNYLNLNHTDVQFEANQKLISMASDKNPKIAITGFCFRAQNVLNNL-----RDENEQRRFLRDTFQDFGWETSKILELM 260
Tet(52)         HEYKLNDLG----AYFSVFSIPNYLNLNNTEVQCEANQKLLSITSDKNRKMAEVAFSFRGQNVLNNV-----RDESEQRRVLRDTFQDFGWEAPRILELM 260
Tet(53)         DEYKLVNLG----VCFGVFSILNYLNLSHTEVQCEANQKLISIWSHKNPKMAEVAFVFRTQNVLNNI-----RNKNEQQQLLRDAFQDFGWEASKILELM 260
Tet(54)         DEYRLVNLA---EAYFSIFSIPNYLNLSHTEVQCETNEKLVSITSDKDPKTAQVAFMFRSQHVWNNL-----RDEHEQMQFLRDIFHDFGWEAQKILELM 261
Tet(55)         DDYHLRNLG----CYISVFSIPNYLQLDHCETLLEAKQKLVSITSDKDSTKAFAGFMFRSSNSPNYI-----RDEASQKDFLRENFTNHGWESNKLLSLM 260
Tet(56)         NEYQLIHLG----AYLSTFTIPNYLGLSHIDLECEANNKLVSINSDNNPEIARAGFMFRSQHLLNDI-----RDEQEQKQFLRDTFRDFGWETQNILNRM 260
Tet(X)          EVEETGTFNIQADIHQPEINCPGFFQLCNGNRLMASHQGNLLFANPNNNGALHFGISFKTPDEWKNQTQVDFQNRNSVVDFLKEFSDWDERYKELI--- 278

aTC residues                                           ●●                                                   ●
CTC residues                                           ●● ●                                                 ●
Tet(47)         SDSDDFYF-DSVSQVKMKSWTKG---RVALLGDAGYCASPISGQGNNLALVGAYVLAGELKQASDNYHQ-AFNRYNELLHPFIEANQKLGVLVNESFLVQ 383
Tet(48)         EESNDFYF-DVATQIKMKSWTKG---RIALVGDSGYCSTALSGQGTTTALVGAYILAGELKAANGNHIT-AFERYNMLLHPFVEANQELGAWINETFLLE 364
Tet(49)         SDSDNFYF-DSVTQVEMKSWTKG---RVALLGDAGYSASPISGQGNNLALVGAYVFAGELKQAGGNYHR-AFSRYNELLHSFVEANQKLGILVNESTLVY 355
Tet(50)         PESDDFYF-DAITQIKMKSWTKG---RIALIGDAAYCPSPLSGQGNNLAFVGAYILAGELKKADGDYIQ-AFTRYNELLHPFVEANQQFGVWVSESFLLK 355
Tet(51)         SDSNDFYF-DSFTQVKMKSWTKG---RVALVGDAGYCASPFPGQGSNQALVGAYIFAGELKQAEGNYHR-AFNRYNELLQPFVEANQKFGVLVNESFLVR 355
Tet(52)         SDSDDFYS-DSFTQVIMKSWTKG---RVALLGDAAYSASPISGQGNNLALVGAYVLAGELKQAGGNYKR-AFDRYNEILRPFIEANQKLGVLVNESFLVR 355
Tet(53)         TGSDDFYF-DSATQVKMKSWTKG---RVALLGDAGYSASPLSGQGNYLALVGAYIFAGELKKADGNYTR-AFSRYNELLHPLVEACHKLGVLVSESFLVP 355
Tet(54)         PNSHDFYF-DSVTQVKMRSWTKG---QVALVGDASYSASPLSGQGNNLALVGAYILAGELKAAEGNYRL-AFNRYNELLHPFVEANQQLGAWVSESFLVP 356
Tet(55)         NDANDFYF-DAIMQVKMKDWTKG---RIALVGDAGYTPSPLSGQGTSLALVGAYILAGELKTATDH-VA-AFARYNELLKPYVEANQAFGVWVSESFLAD 354
Tet(56)         PESNDFYF-DAITQVKMNSWTKG---RIALVGDAGYCPSPLSGQGNNLAFVGAYILAGELKVANGNYTR-AFTRYNALLRSFVDANQKFGVWVSESFLVK 355
Tet(X)          HTTLSFVGLATRIFPLEKPWKSKRPLPITMIGDAAHLMPPFAGQGVNSGLVDALILSDNLADGKFNSIEEAVKNYEQQMFIYGKEAQEESTQNEIEMFKP 378

aTC residues                    ●
CTC residues                    ●
Tet(47)         -DEVSKEVAEERSNKIMEEVKIVSNMISLPDYE--- 415
Tet(48)         -DAVSKEAVEARTDNIIKKISAISNVIKLPEYSAYK 399
Tet(49)         -GEVSQEVAEERSNKIMQEVEIAANMISLPNYE--- 387
Tet(50)         DDEVSKEIAEARSNKILAMIKSVSNSINLPQYE--- 388
Tet(51)         -DEVSKEVAEERSNKIMQEIKIVSNMISLPNYE--- 387
Tet(52)         -DEVSKEVAEERSNNIMEQVKIASNMIVLQDYANPH 390
Tet(53)         -DEVSKEVAEERSNKILQEVKIVSNMISLPEYE--- 387
Tet(54)         -DAVSKEVAEERSNRILQKVQIISNAIKLPEYE--- 388
Tet(55)         -EPLSAEQAEERNNIVLGIMKKATHAIELPEY---- 385
Tet(56)         -DEVSKEIAEERSNKILAMIKSISNGITLPQYESS- 389
Tet(X)          DFTFQQLLNV-------------------------- 388
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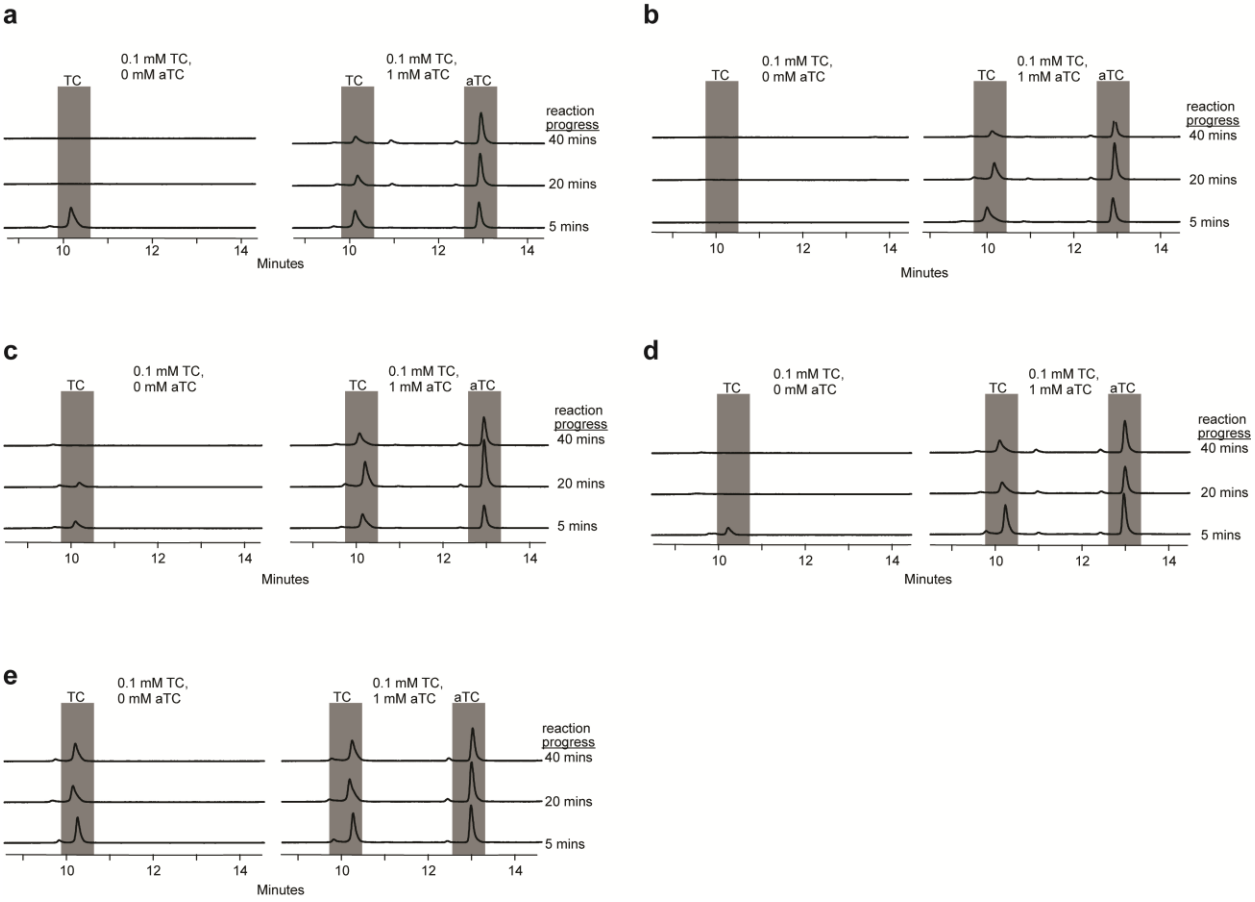
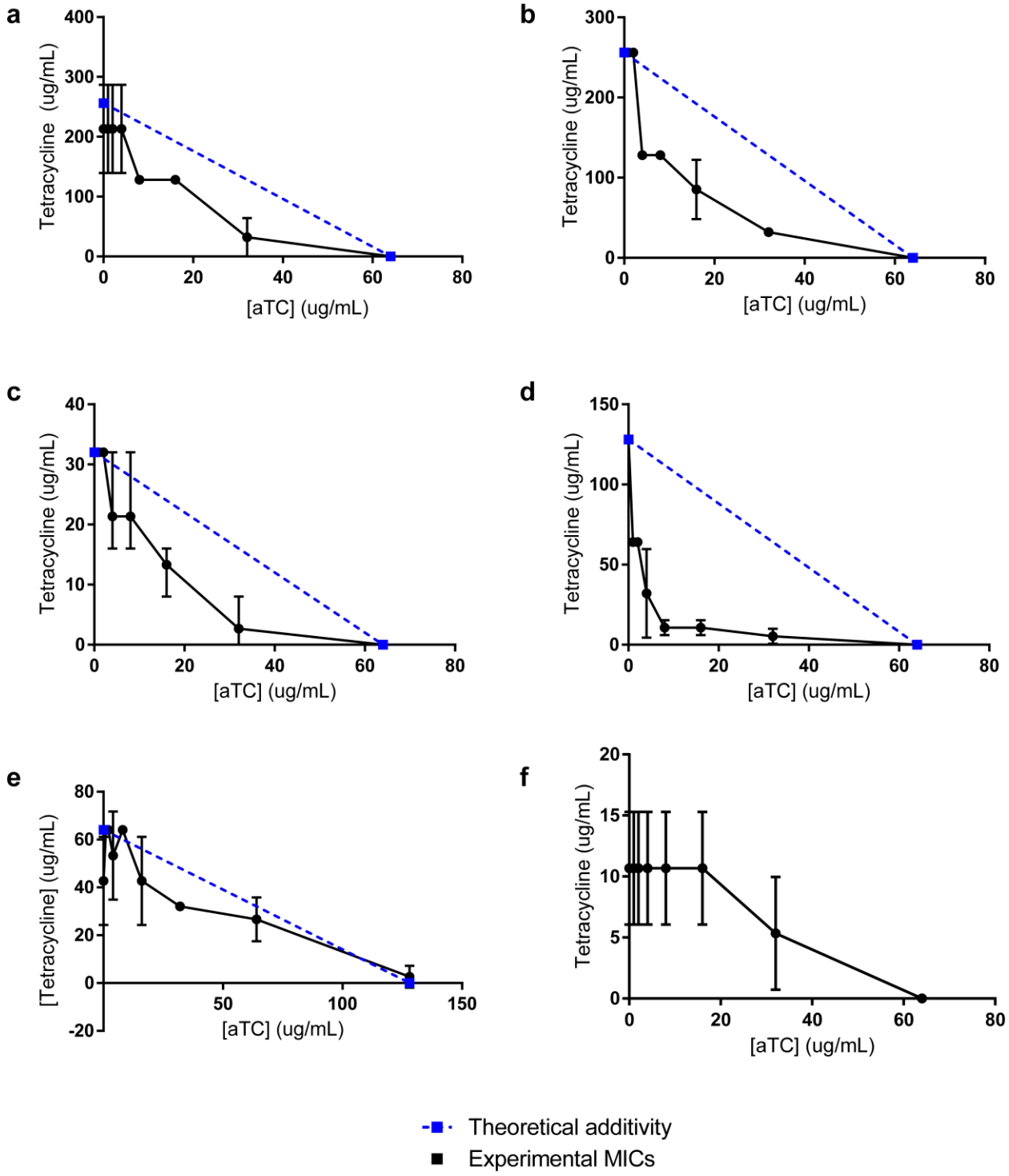
Supplementary Figure 8 | Anhydrotetracycline prevents enzymatic degradation of tetracycline. HPLC chromatograms indicate complete consumption of 0.1 mM tetracycline (TC) over the period assayed by Tet(50) **(a)**, Tet(51) **(b)**, Tet(55) **(c)**, and Tet(X) **(d)**, but not in the no enzyme control **(e)**. 1 mM anhydrotetracycline (aTC) is sufficient to decrease or prevent tetracycline degradation.

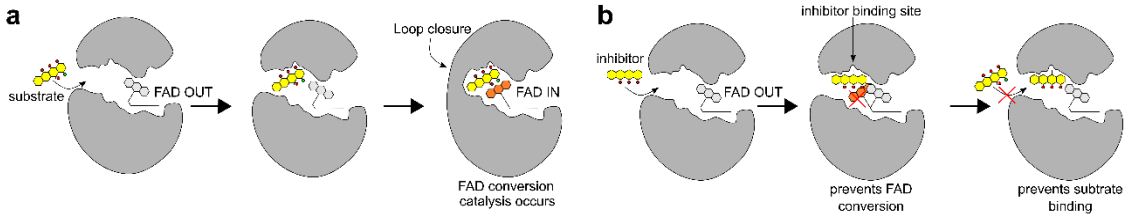


Supplementary Figure 9 | Anhydrotetracycline synergizes with tetracycline to kill *E. coli* expressing *tet*(50,51,55,56) but not *tet*(X). Anhydrotetracycline exhibits synergy with tetracycline against *E. coli* expressing *tet*(50) **(a)**, *tet*(51) **(b)**, *tet*(55) **(c)**, and *tet*(56) **(d)**, but not *tet*(X) **(e)** or empty vector control **(f)**. Dashed blue lines indicate the theoretical concentrations of additive interactions.



Supplementary Figure 10 | Model for binding dynamics, substrate plasticity, and inhibition of tetracycline-inactivating enzymes

a, Substrate (e.g. chlortetracycline) can enter and bind the active site of a tetracycline destructase, resulting in a conformational switch from FAD OUT (grey) to FAD IN (orange) and closure of the substrate site.
b, A mechanistic inhibitor (e.g. anhydrotetracycline) enters and binds the active site, but sterically prevents the FAD cofactor from switching from the OUT to IN conformation and thereby preventing catalysis. Further, it can act synergistically to competitively prevent substrate from binding.



Supplementary Table 1 | Data collection and refinement statistics

	Tet(55) Native	Tet(55) SeMet	Tet(50)	Tet(51)	Tet(56)	Tet(50) + chlortetracycline	Tet(50) + anhydrotetracycline
Data collection							
Space group	$P2_12_12$	$P2_12_12$	$P2_12_12_1$	$P2_1$	$P2_12_12$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions							
a, *b*, *c* (Å)	64.74, 124.43, 45.73	65.14, 123.98, 45.75	50.94, 107.61, 152.48	83.49, 81.69, 127.31	76.49, 114.02, 94.81	51.10, 107.22, 152.63	50.99, 107.37, 152.79
α, β, γ (º)	90, 90, 90	90, 90, 90	90, 90, 90	90, 96.790, 90	90, 90, 90	90, 90, 90	90, 90, 90
		Peak					
Wavelength	1.018211	0.976289	1.000029	1.000028	1.000032	1.000031	1.000031
Resolution (Å)	20 - 2.00 (2.10-2.00)	20 - 1.90 (2.00 - 1.90)	20 - 2.10 (2.20 - 2.10)	20 - 1.85 (1.95 - 1.85)	20 - 3.30 (3.40 - 3.30)	20 - 1.75 (1.85 - 1.75)	20 - 2.25 (2.35 - 2.25)
R_{meas}	11.0% (96.4%)	6.4% (59.7%)	10.8% (71.6%)	9.7% (79.7%)	13.2% (119.2%)	8.6% (86.5%)	11.5% (68.7%)
$I/\sigma I$	16.14 (2.24)	17.45 (2.57)	15.07 (2.47)	11.93 (1.90)	10.76 (1.16)	19.31 (2.35)	13.57 (2.37)
Completeness (%)	99.7 (98.8)	99.6 (98.0)	98.1 (99.3)	99.7 (99.6)	98.5 (94.0)	98.7 (98.1)	98.6 (92.7)
Redundancy	7.17 (6.85)	3.79 (3.59)	5.70 (5.55)	3.77 (3.67)	3.60 (3.22)	7.42 (7.37)	4.93 (4.92)
Refinement							
Resolution (Å)	20 - 2.00		20 - 2.10	20 - 1.85	20 - 3.30	20 - 1.75	20 - 2.25
No. reflections	25,629		48,794	144,460	12,803	84,325	39,793
R_{work}/ R_{free}	19.25/ 23.86		22.81/26.23	16.67/ 19.99	23.96/ 29.55	17.80/ 21.90	20.68/ 25.45
No. atoms							
Protein	3,284		6,642	13,097	5,819	6,733	6,698
Ligand/ion	5		136	256	116	181	182
Water	187		376	942	0	444	337
B-factors							
Protein	30.24		29.76	27.82	97.42	23.42	30.35
Ligand/ion	28.36		35.73	17.93	82.54	29.83	38.66
Water	30.51		29.57	32.19		26.88	28.68
R.m.s deviations							
Bond lengths (Å)	0.005		0.003	0.007	0.002	0.011	0.003
Bond angles (º)	0.797		0.667	1.224	0.645	1.273	0.742

Supplementary Table 2 | Kinetic parameters for Tet(50,55,56,X). Data are represented as mean ± s.e.m of three technical replicates.

	Tetracycline			**Chlortetracycline**		
	K_m (μM)	**k_{cat} (min^{-1})**	**k_{cat}/K_m ($\mu M^{-1}min^{-1}$)**	**K_m (μM)**	**k_{cat} (min^{-1})**	**k_{cat}/K_m ($\mu M^{-1}min^{-1}$)**
Tet(50)	17 ± 3.6	4.3 ± 0.23	0.25	6.3 ± 2.0	3.5 ± 0.25	0.55
Tet(55)	4.6 ± 1.6	1.8 ± 0.15	0.41	6.0 ± 1.3	2.9 ± 0.04	0.48
Tet(56)	7.7 ± 1.6	6.4 ± 0.31	0.83	3.7 ± 1.1	6.6 ± 0.39	1.8
Tet(X)	11 ± 2.6	0.67 ± 0.04	0.06	7.9 ± 2.7	0.90 ± 0.07	0.11

Supplementary Table 3 | Chlortetracycline minimum inhibitory concentrations (MIC) for *E. coli* expressing tetracycline inactivating enzymes

	MIC (µg/mL)
empty vector	16
tet(50)	256
tet(51)	512
tet(55)	256
tet(56)	512
tet(X)	256

Supplementary Table 4 | HPLC peak height ratios. Ratios of tetracycline (TC) to anhydrotetracycline (aTC) from chromatograms in Figure 6 and Supplementary Figure 8.

Enzyme	Reaction progress (mins)	TC/aTC peak height ratio
No Enzyme	5	0.639
No Enzyme	20	0.579
No Enzyme	40	0.578
Tet(50)	5	0.670
Tet(50)	20	0.344
Tet(50)	40	0.249
Tet(51)	5	0.613
Tet(51)	20	0.456
Tet(51)	40	0.430
Tet(55)	5	0.668
Tet(55)	20	0.524
Tet(55)	40	0.441
Tet(56)	5	0.488
Tet(56)	20	0.472
Tet(56)	40	0.472
Tet(X)	5	0.731
Tet(X)	20	0.435
Tet(X)	40	0.387

Supplementary Table 5 | Relevant strains, plasmid, and primers employed in this study

Strains, Plasmids, Primers		**Reference or source**
Strains		
Lp02	*Legionella pneumophila* Philadelphia I	(Berger and Isberg, 1993)
JV595	*Legionella longbeachae*	ATCC 33462
JV8858	*Legionella longbeachae* Δ*tet*(56)	This study
JV8861	JV595 + pJB7207	This study
JV8864	JV595 + pJB1625	This study
JV8868	JV8858 + pJB7207	This study
JV8870	JV8858 + pJB1625	This study
JV8874	Lp02 + pJB7207	This study
JV8876	Lp02 + pJB1625	This study
Plasmids		
pSR47S	Suicide plasmid R6K suicide vector (KanR *sacB*)	(Merriam et al., 1997)
pJB1625	Complementing vector (CmR version of pJB908)	(Sexton et al., 2004)
pJB7204	Δ*tet*(56) suicide plasmid	This study
pJB7207	*tet*(56) complementing clone	This study
Primers		
JVP2910	GCAGCGGCCGCGCCAATGACGAGAATTTTGATATTTTTAGAC	
JVP2911	GCAGCGGCCGCCAATTTCGAATGGGATTACCTTACCTC	
JVP2912	GCAGAGCTCGCTTAATGATATCAAGATTAATACAATTCCAATCC	
JVP2913	GCAGTCGACGCTCAATTGTATGTTCGTTATGAAGATGGG	
JVP2921	CCAGGATCCTAAGAGGAGAAATTAACTATGTCTAAAAATATCAAAATTCTCGTC	
JVP2922	CCAGTCGACGTCCACTATGATGATTCATATTGAGG	