Supplementary material

Putative dioxygen binding sites and recognition of tigecycline and minocycline in the tetracycline-degrading monooxygenase TetX

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PDB References: Monoxygenase TetX, complex with minocycline, 4a99; complex with tigecycline, 4a6n; complex with xenon, 4guv.

Molecular Mechanics Parameters for 7-chlortetracycline and $FADH_2$

The 7-chlortetracycline parameters were obtained using ATB (Malde *et al.*, 2011), but its partial charges were further refined by calculating the electronic potential with Gaussian09 (Frisch *et al.*, 2009), using the B3LYP hybrid functional and the 6-31G(d) basis set, and fitting the atomic charges with RESP (Bayly *et al.*, 1993). $FADH_2$ parameters were derived by combining the GROMOS96 parameters (van Gunsteren *et al.*, 1996) of fully reduced FMN (for the flavin mononucleotide part) and NADH (for the adenosine part).

Details on Molecular Dynamics Simulations

The simulations were performed at 298.15 K and 1 atm, with an integration time step of 0.002 ps. The temperature and pressure were kept constant with Berendsen coupling baths (Berendsen *et al.*, 1984) with separate temperature coupling for solutes and solvent. In the production simulations, the pressure coupling constant was 0.5 ps and the heat coupling constant was 0.1 ps. The van der Waals interactions were considered up to 14 Å and the electrostatic interactions beyond 9 Å were treated with a smooth particle mesh Ewald method (Darden *et al.*, 1993, Essmann *et al.*, 1995). The neighbour lists were updated every 10 steps. All bonds were constrained to their equilibrium lengths with the P-LINCS algorithm (Hess, 2008) except the water molecules, which were kept rigid with the SETTLE algorithm (Miyamoto & Kollman, 1992).

Figure S4 shows the minimum distance of dioxygen to C4a of $FADH_2$ throughout simulation time for each replicate simulation.

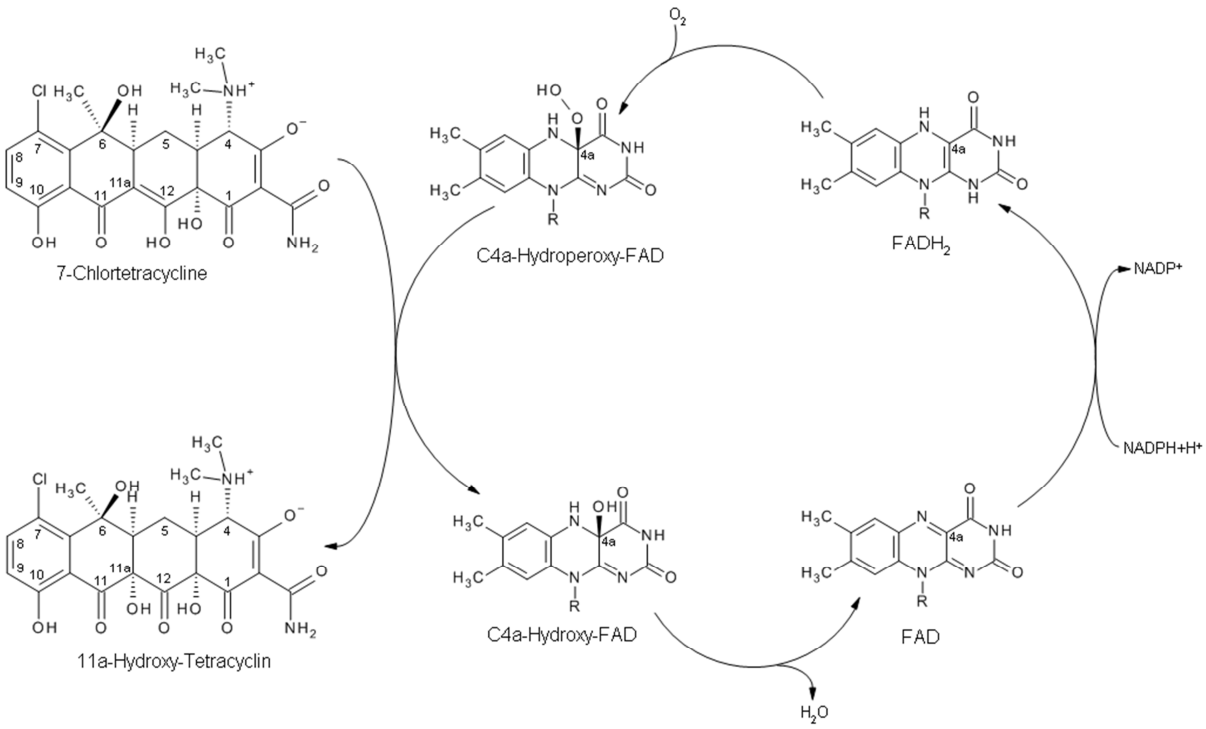

Figure S1
Reaction pathway of tetracycline hydroxylation by TetX. Molecular dioxygen is activated by binding to $FADH_2$ forming the C4a-hydroperoxide which hydroxylates tetracyclines at C11a. In TetX the molecular oxygen will bind from the viewing direction to C4a to form the hydroperoxide. This is the *re*-side of the isoalloxazine of FAD/$FADH_2$.

Nomenclature of the *re*/*si* System:
The enantiotopic faces of a trigonal (carbon-)atom are named *re* and *si* using the *Cahn-Ingold-Prelog*-System (Hanson, 1966).

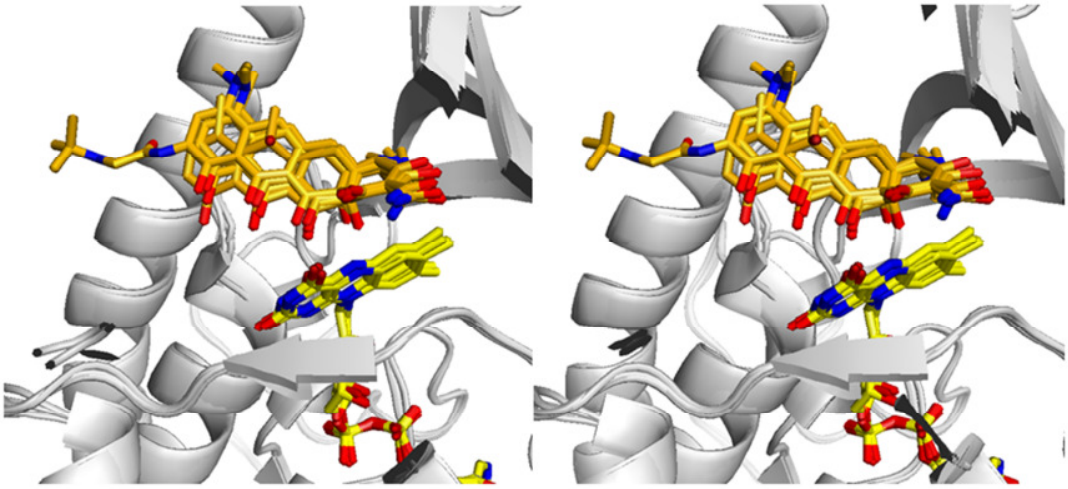

Figure S2

Stereo view of the substrate binding site of TetX. Superposition of the TetX complexes with 7-chlortetracycline, 7-iodtetracycline, minocycline and tigecycline (PDB entries 2y6r, 2y6q, 4a99, 4a6n). Side chains are omitted for clarity. Colour code of FAD and tetracyclines according to Figure S3. The isoalloxazine of FAD and the A and B rings of the tetracyclines are almost in the same position due to direct hydrogen bonds, whereas the C and D ring vary slightly more because of their less directed hydrophobic interactions with TetX.

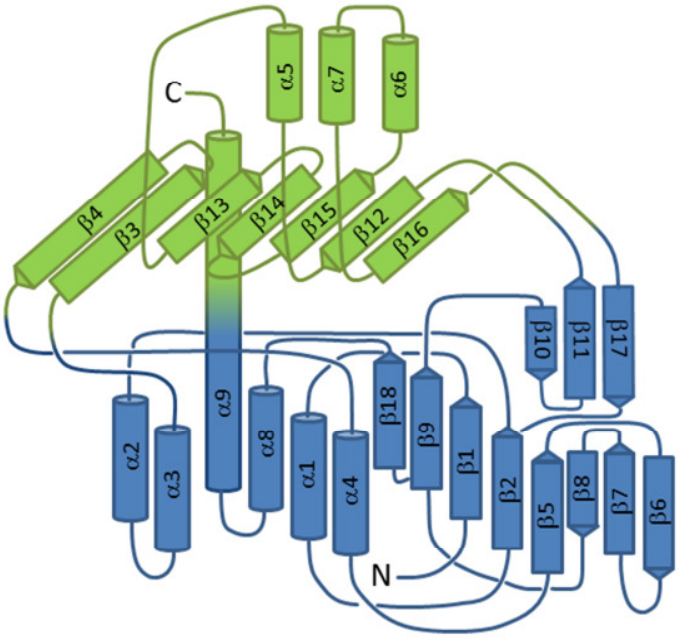

Figure S3
Topology diagram of the monooxygenase TetX. Secondary structure arrangement of the FAD-binding domain (blue) and the substrate binding domain (green). The enzyme (383 amino acid residues) is composed of two domains. The FAD-binding domain exhibits a Rossmann fold binding the adenosine monophosphate component, which is linked to the flavin mononucleotide containing the catalytically active isoalloxazine moiety of the coenzyme. The substrate-binding domain with a 7-stranded β-sheet is positioned like a shield on top of the FAD-binding domain covered by α-helices. The long C-terminal α-helix (10 turns) stabilizes the association of the two domains (Volkers *et al.*, 2011).

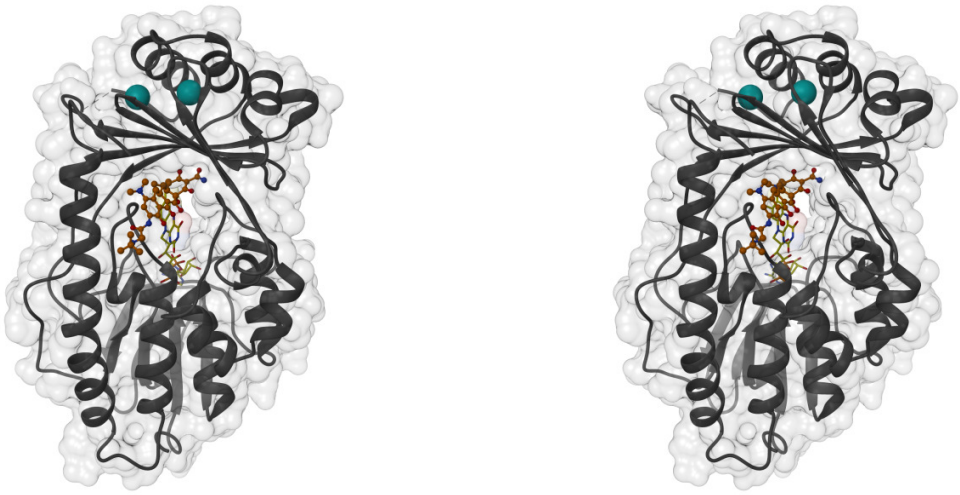

Figure S4
Stereo representation of TetX:tigecycline complex with transparent surface of the protein. The superposition on main chain atoms (N, Cα, C, O) of the TetX:Xe complex revealed an r.m.s. deviation of only 0.33 Å. The Xe positions are shown to highlight the β-sheet shielding of the substrate binding-site. The orientation is comparable to Figure S2 with ball-and-stick model of tigecycline with orange carbon atoms, FAD shown as sticks with yellow carbon atoms.

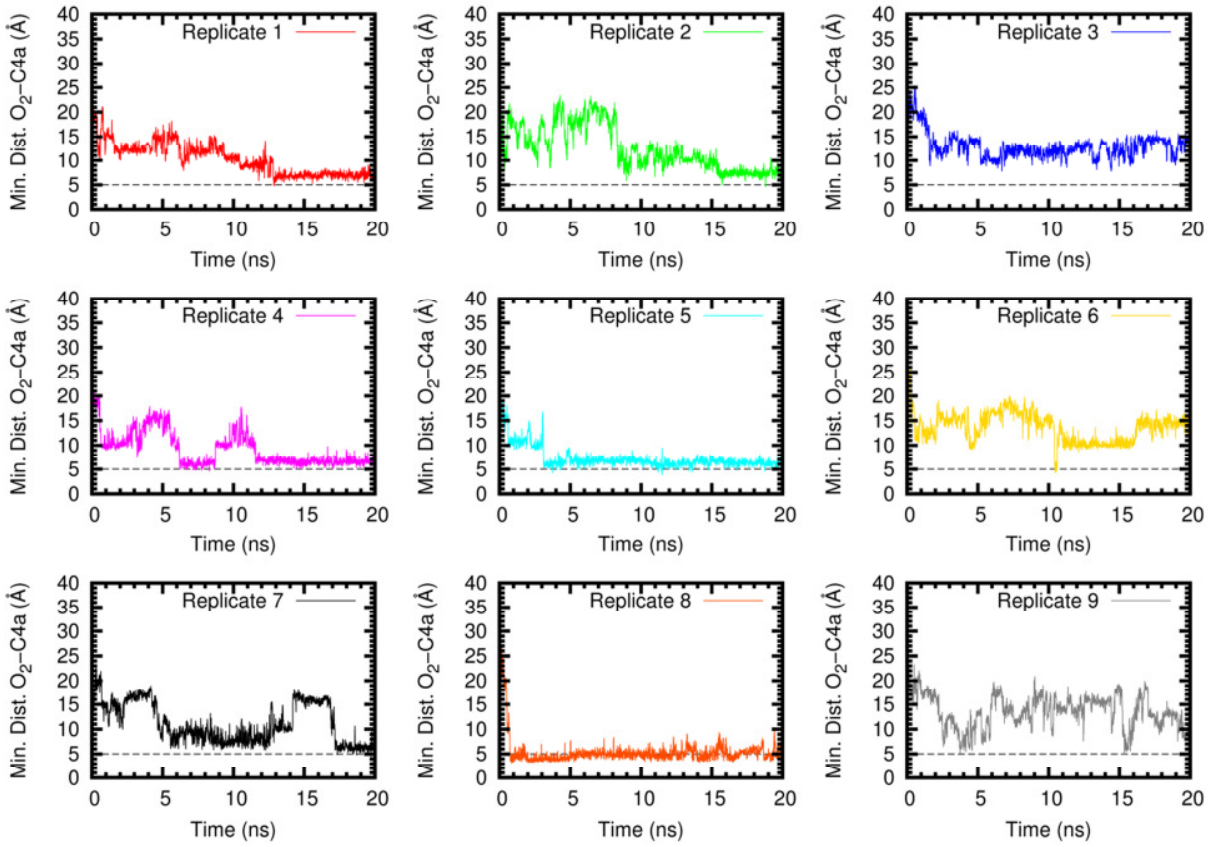

Figure S5

Minimum distance of dioxygen to C4a of $FADH_2$ throughout simulation time for each replicate simulation. The data are plotted every 10 ps. In most replicate simulations, the dioxygen reaches a distance around 5 Å.

Table S1. Additional parameters of data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

	TetX:MTC	TetX:TTC	TetX:Xe
Beamline	BESSY II/14.2	BESSY II/14.2	BESSY II/14.2
Detector	CCD, MX-225	CCD, MX-225	CCD, MX-225
Wavelength (Å)	0.91841	0.91841	1.5
Crystal-to-detector distance (mm)	210	240	180
Total rotation and range per image (°)	360/1	360/1	360/1, 360/1
Exposure time per image (s)	5	4	5
Space group	*P*1	*P*1	*P*1
Unit cell parameters *a*, *b*, *c* (Å); α, β, γ (°)	 68.87, 80.33, 86.63; 110.82, 90.27, 93.39	 68.88, 80.79, 87.65; 110.84, 89.98, 93.63	 68.71, 80.14, 87.50; 111.04, 90.06, 93.29
Resolution (Å)	80.94-2.18 (2.31-2.18)	81.90-2.30 (2.42-2.30)	81.64-2.73 (2.80-2.73)
No. of measured reflections	324583	284421	294757
No. of unique reflections	86443	75168	85826 *
Mosaicity (°)	0.43	1.14	0.66

* processed keeping Friedel mates separate.

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