From binding to breakdown: biophysical and molecular insights into fluoroquinolone induced F-actin perturbation

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Supplementary Figures

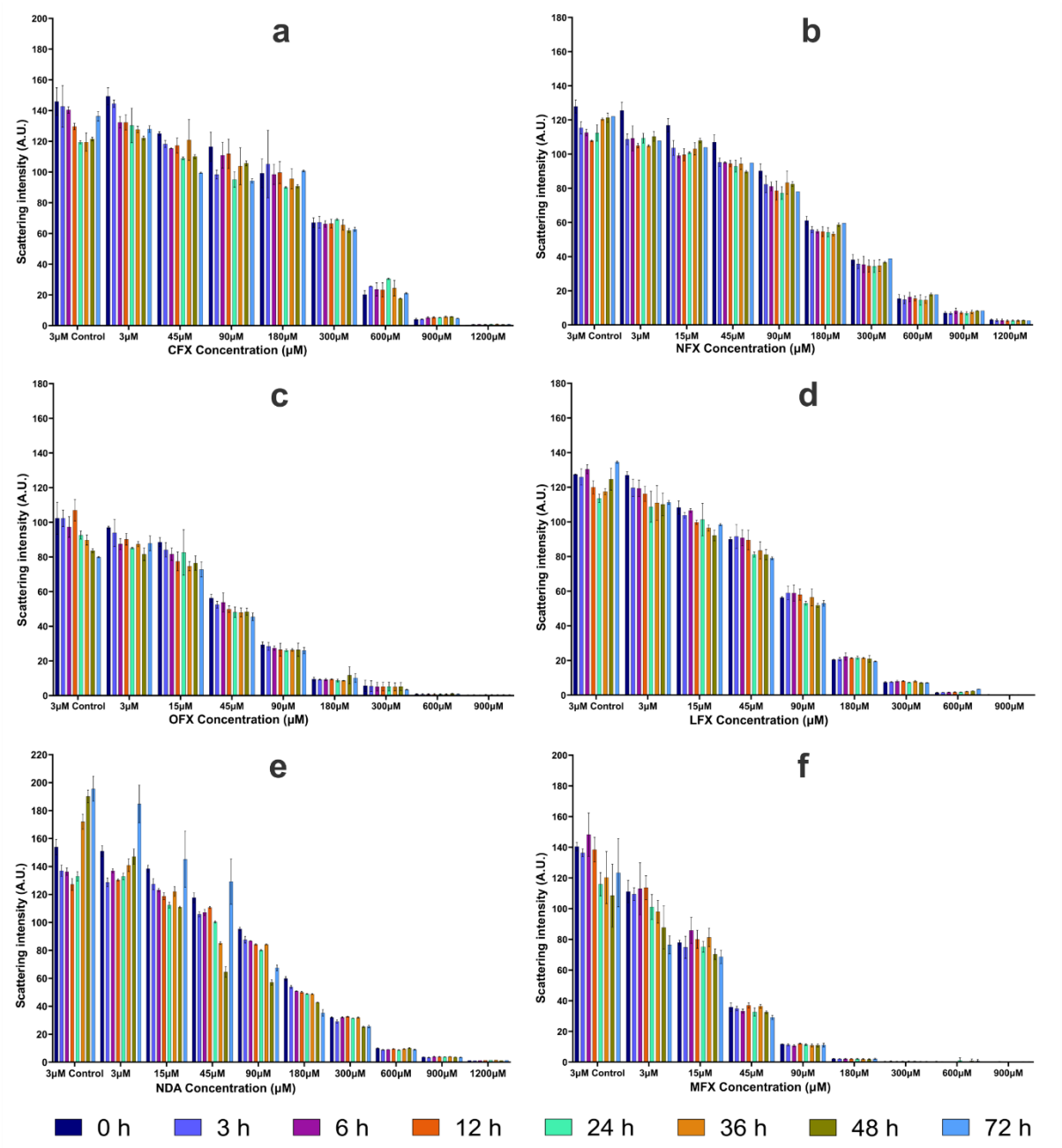

Fig.S1. F-actin disruption assay using Right Angle Light Scattering (RALS) measurements. F-actin treated with (a) CFX; (b) NFX; (c) OFX; (d) LFX; (e) NDA; (f) MFX at various concentrations (3-1200 μM) for 72 hours. The different colours bars represent measurement time points. Data represents mean and the standard error of mean (mean ± SEM), n=3.

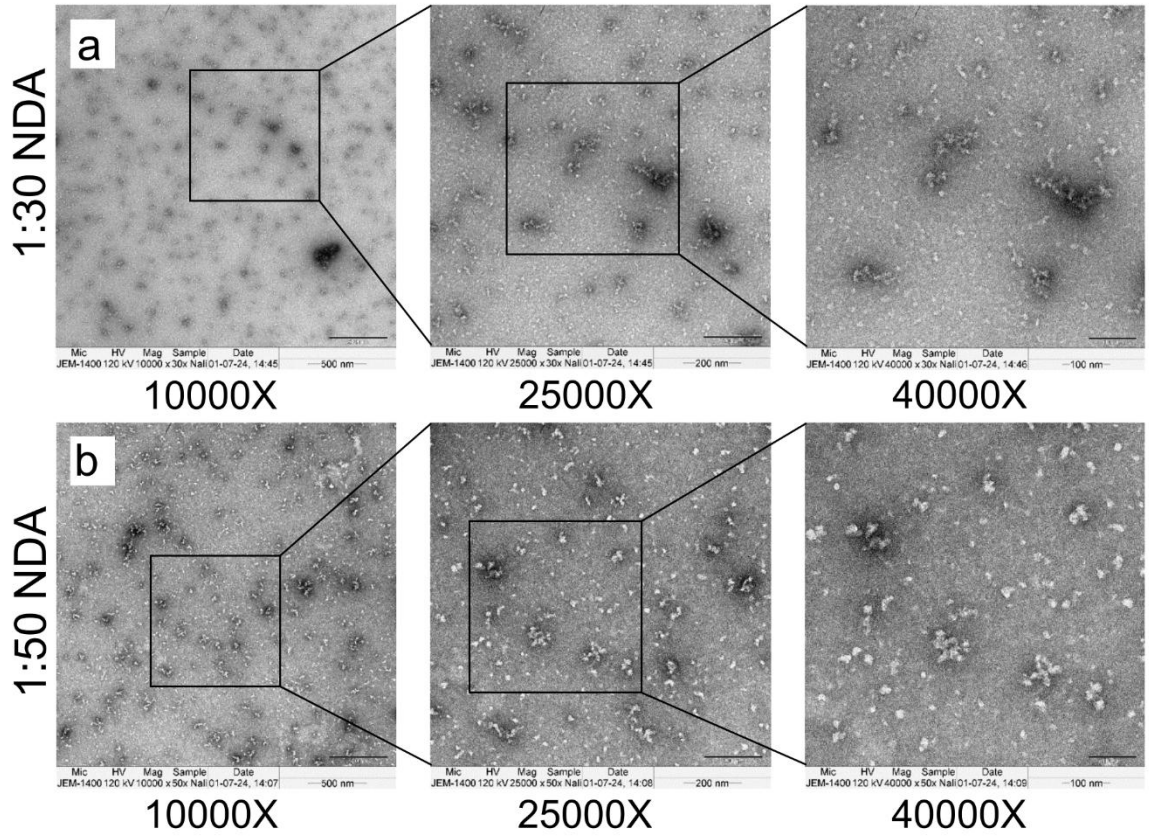

Fig.S2. NDA-induced morphological changes in F-actin visualized by TEM. (a) F-actin treated with NDA at a 1:30 molar ratio; (b) F-actin treated with NDA at a 1:50 molar ratio. Images were captured at 10,000× (scale bar: 500 nm), 25,000× (scale bar: 200 nm), and 40,000× (scale bar: 100 nm).

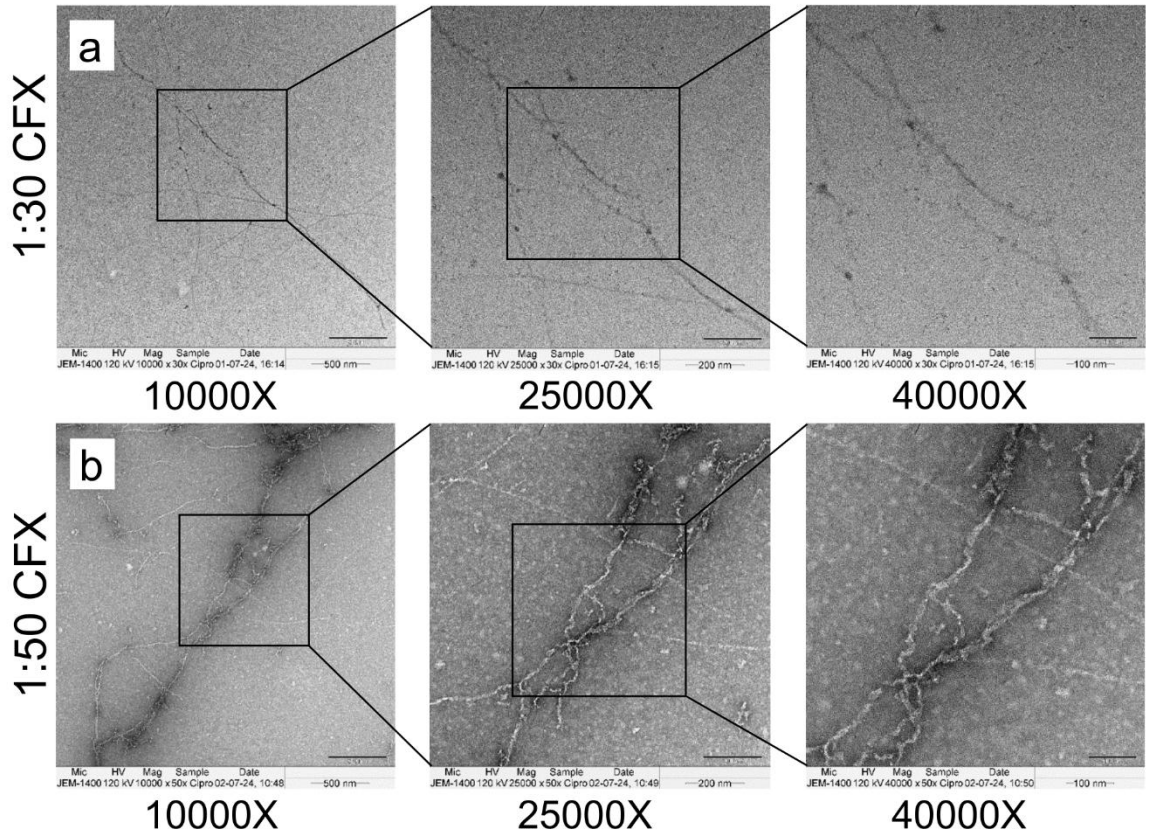

Fig.S3. CFX-induced morphological changes in F-actin visualized by TEM. (a) F-actin treated with CFX at a 1:30 molar ratio; (b) F-actin treated with CFX at a 1:50 molar ratio. Images were captured at 10,000× (scale bar: 500 nm), 25,000× (scale bar: 200 nm), and 40,000× (scale bar: 100 nm).

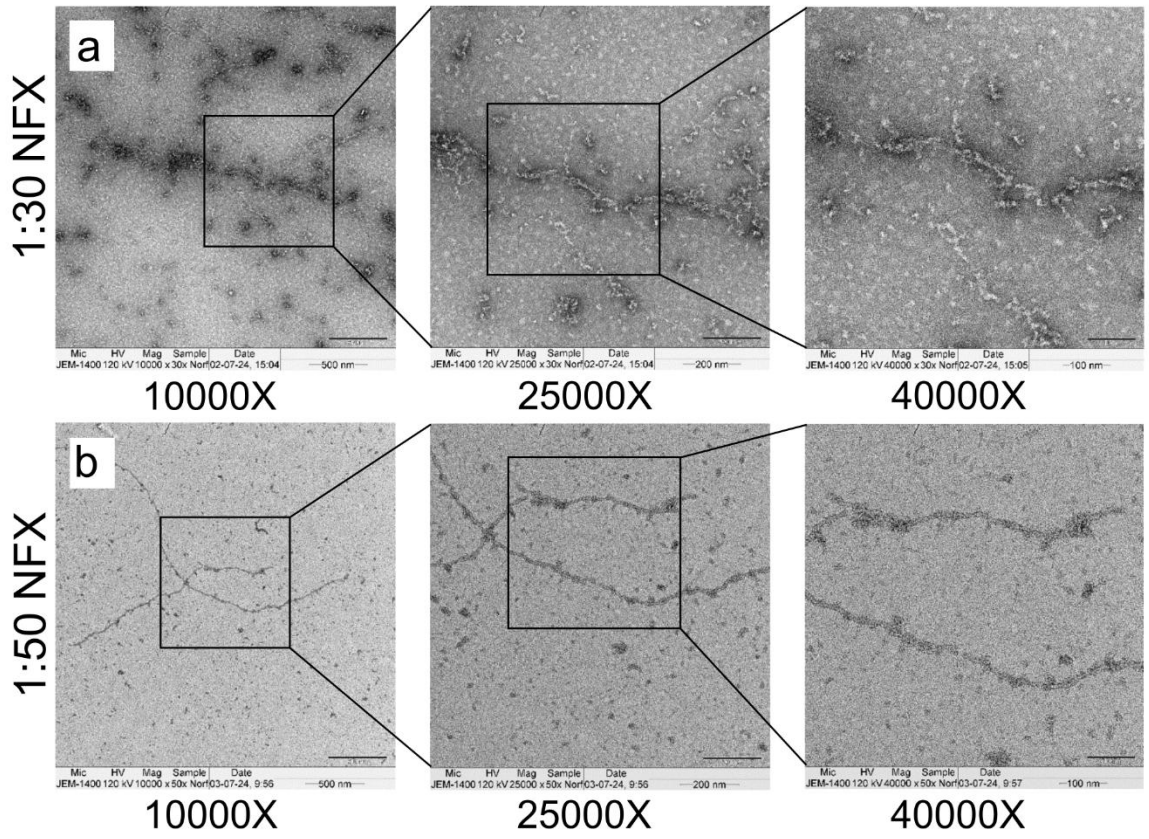

Fig.S4. NFX-induced morphological changes in F-actin visualized by TEM. (a) F-actin treated with NFX at a 1:30 molar ratio; (b) F-actin treated with NFX at a 1:50 molar ratio. Images were captured at 10,000× (scale bar: 500 nm), 25,000× (scale bar: 200 nm), and 40,000× (scale bar: 100 nm).

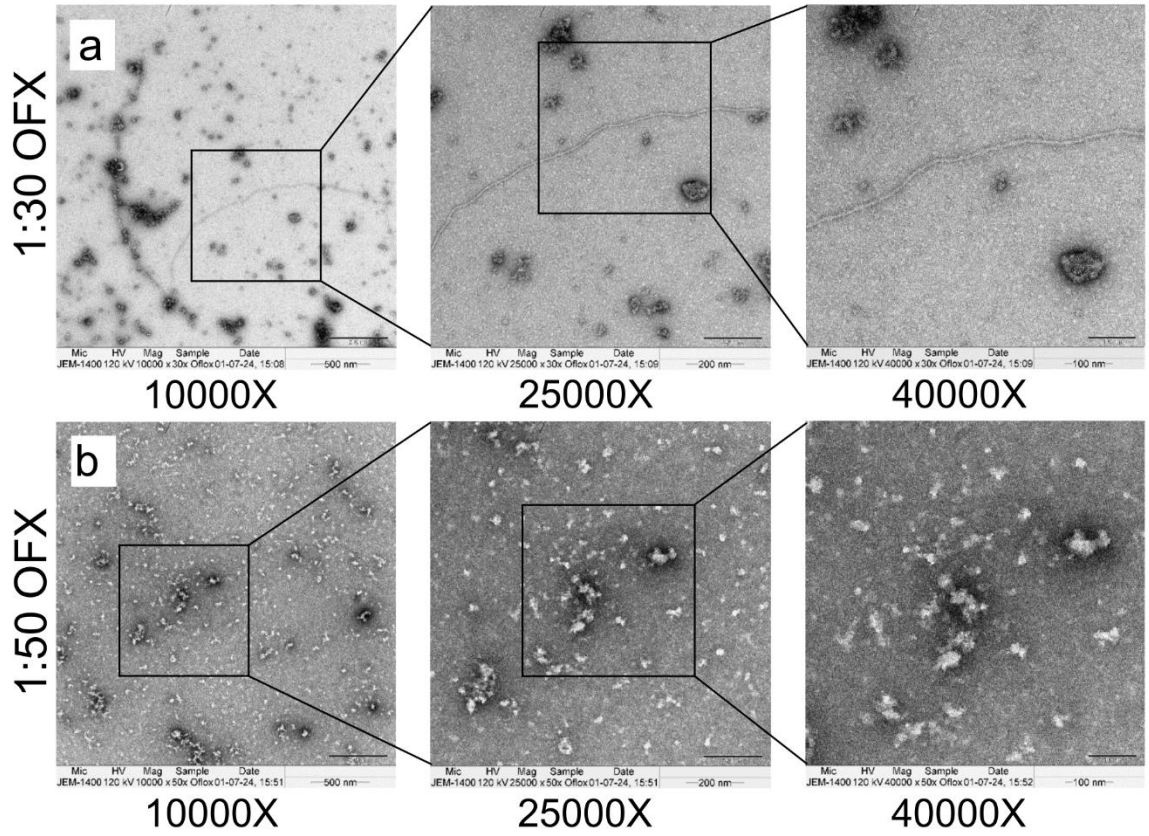

Fig.S5. OFX-induced morphological changes in F-actin visualized by TEM. (a) F-actin treated with OFX at a 1:30 molar ratio; (b) F-actin treated with OFX at a 1:50 molar ratio. Images were captured at 10,000× (scale bar: 500 nm), 25,000× (scale bar: 200 nm), and 40,000× (scale bar: 100 nm).

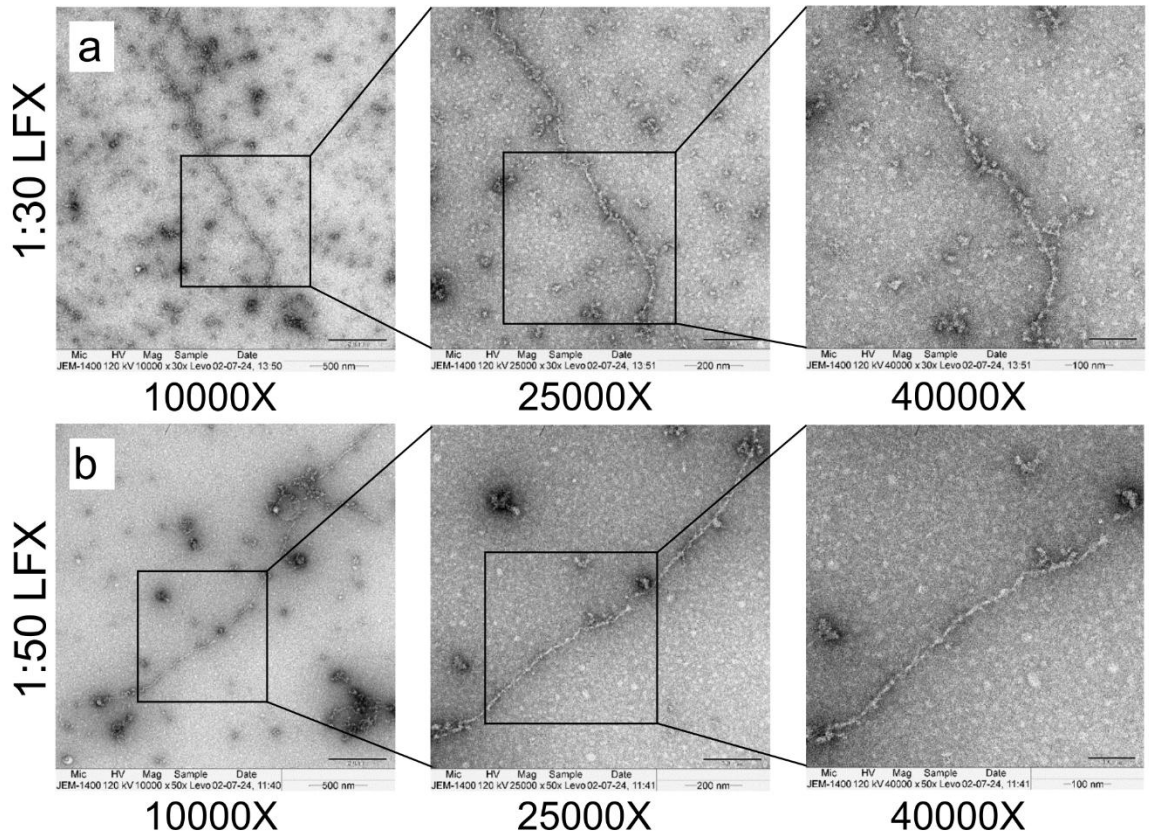

Fig.S6. LFX-induced morphological changes in F-actin visualized by TEM. (a) F-actin treated with LFX at a 1:30 molar ratio; (b) F-actin treated with LFX at a 1:50 molar ratio. Images were captured at 10,000× (scale bar: 500 nm), 25,000× (scale bar: 200 nm), and 40,000× (scale bar: 100 nm).

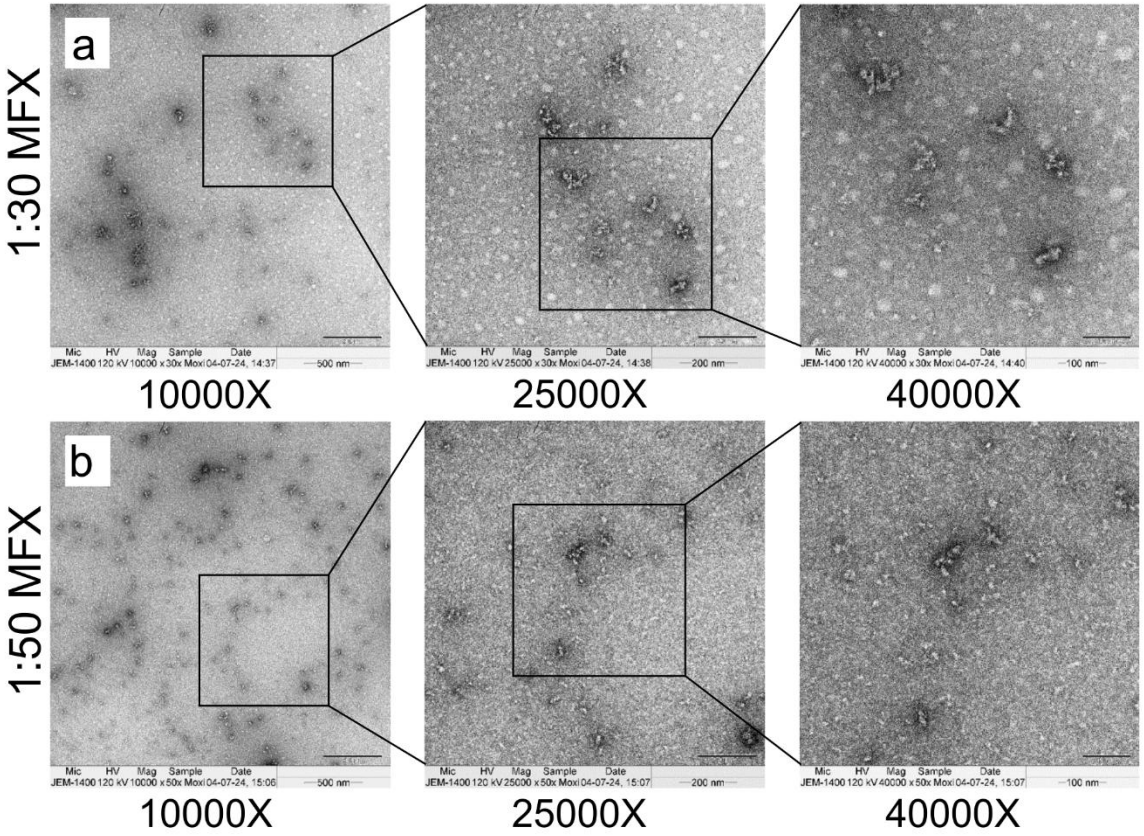

Fig.S7. MFX-induced morphological changes in F-actin visualized by TEM. (a) F-actin treated with MFX at a 1:30 molar ratio; (b) F-actin treated with MFX at a 1:50 molar ratio. Images were captured at 10,000× (scale bar: 500 nm), 25,000× (scale bar: 200 nm), and 40,000× (scale bar: 100 nm).

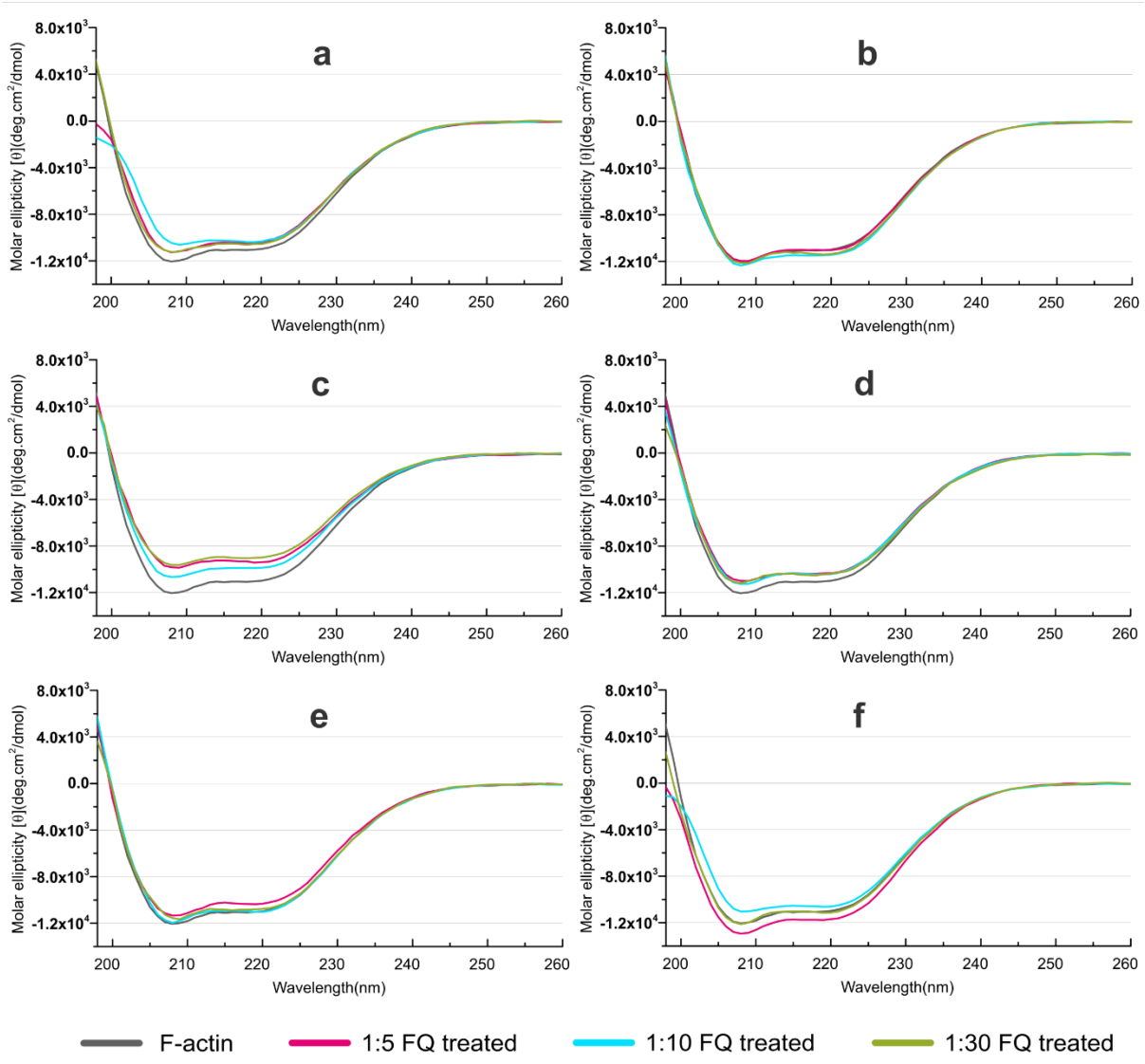

Fig.S8. CD spectra of F-actin treated with (a) CFX; (b) NFX; (c) OFX; (d) LFX; (e) NDA; (f) MFX titrated at 1:5 (red curve), 1:10 (cyan curve), and 1:30 (olive curve) molar ratios. Untreated F-actin is shown as grey curve.

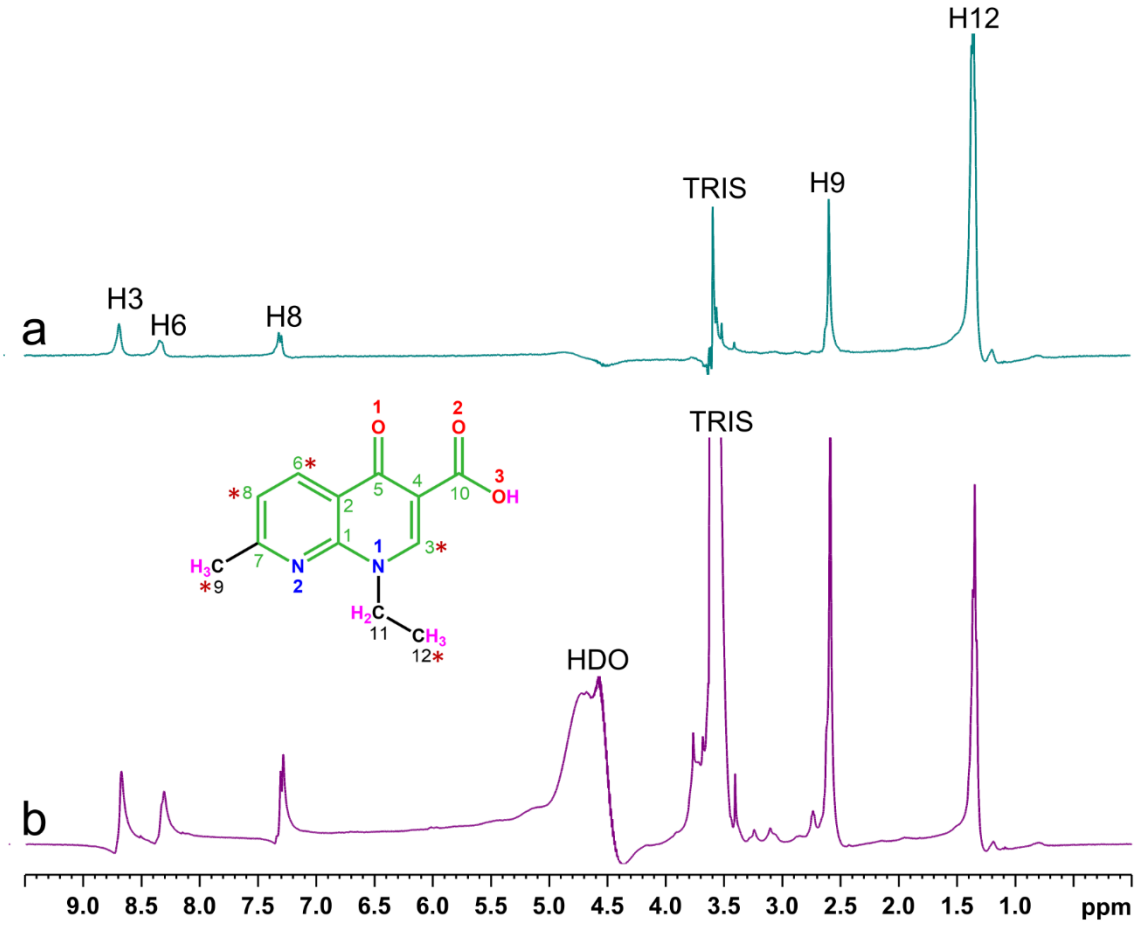

Fig.S9. (a) STD spectrum of NDA in presence of actin; (b) Reference spectrum (off resonance) of NDA in presence of actin. The compound was added to 2mM final concentration to result in a 50-fold excess of the ligand.

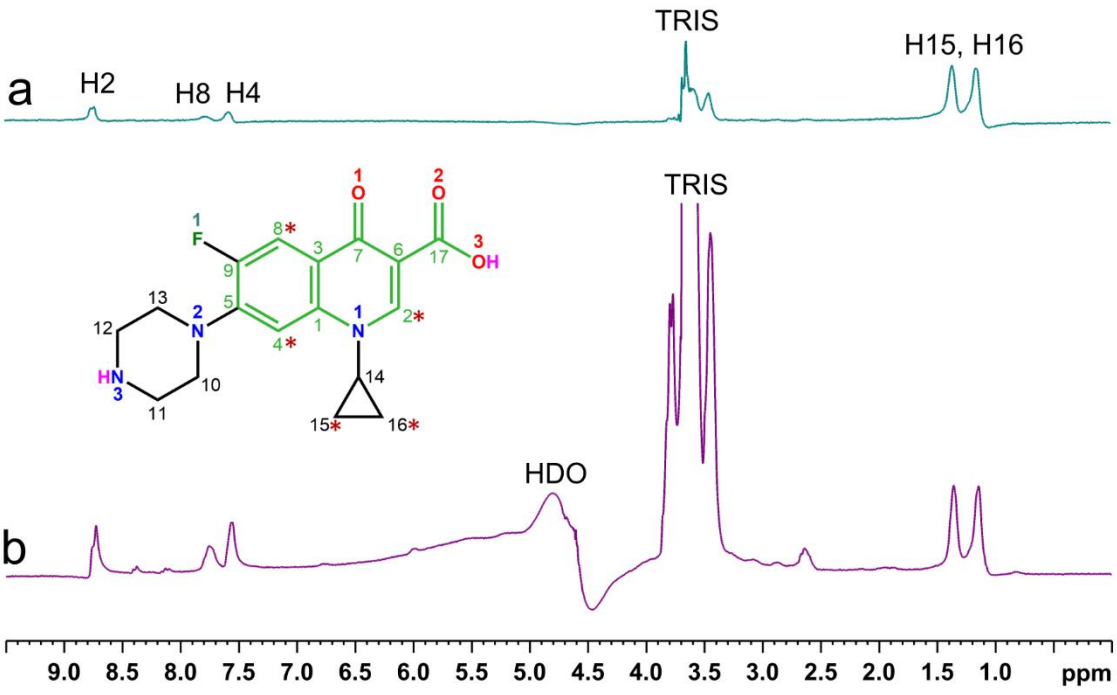

Fig.S10. (a) STD spectrum of CFX in presence of actin; (b) Reference spectrum (off resonance) of CFX in presence of actin. The compound was added to 2mM final concentration to result in a 50-fold excess of the ligand.

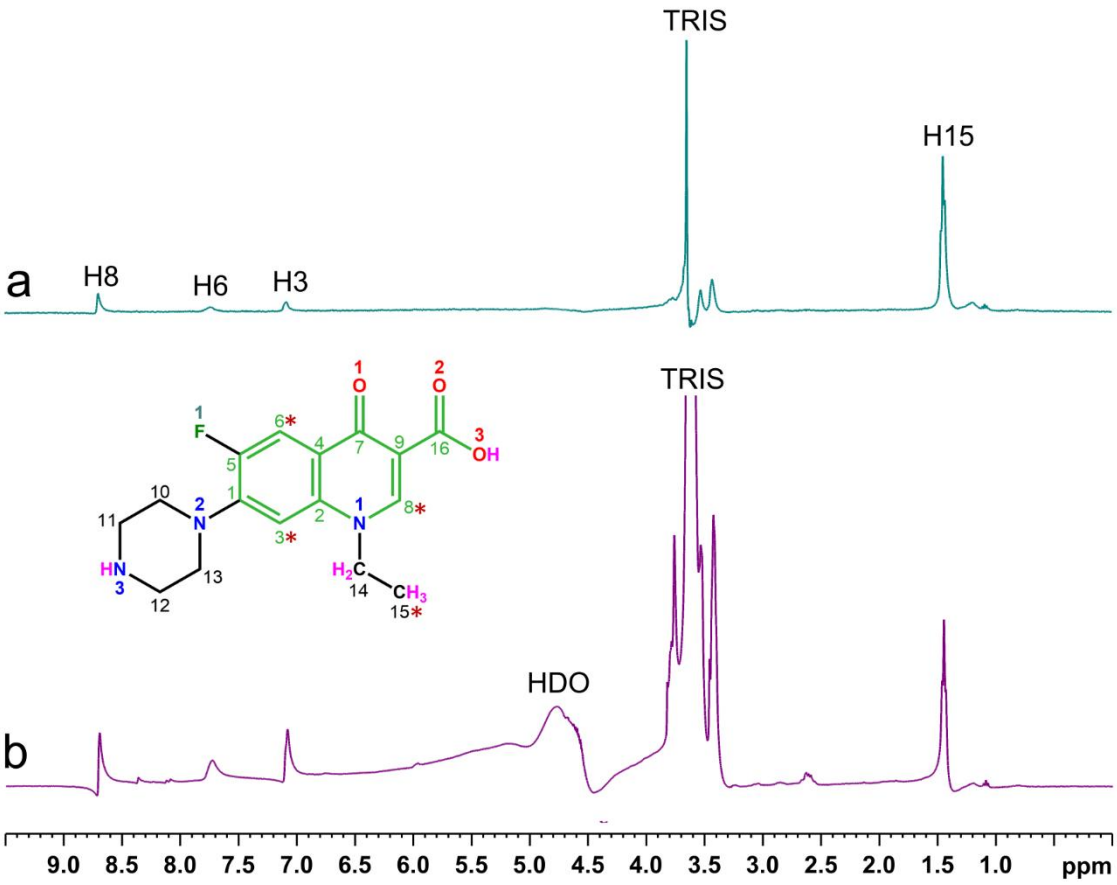

Fig.S11. (a) STD spectrum of NFX in presence of actin; (b) Reference spectrum (off resonance) of NFX in presence of actin. The compound was added to 2mM final concentration to result in a 50-fold excess of the ligand.

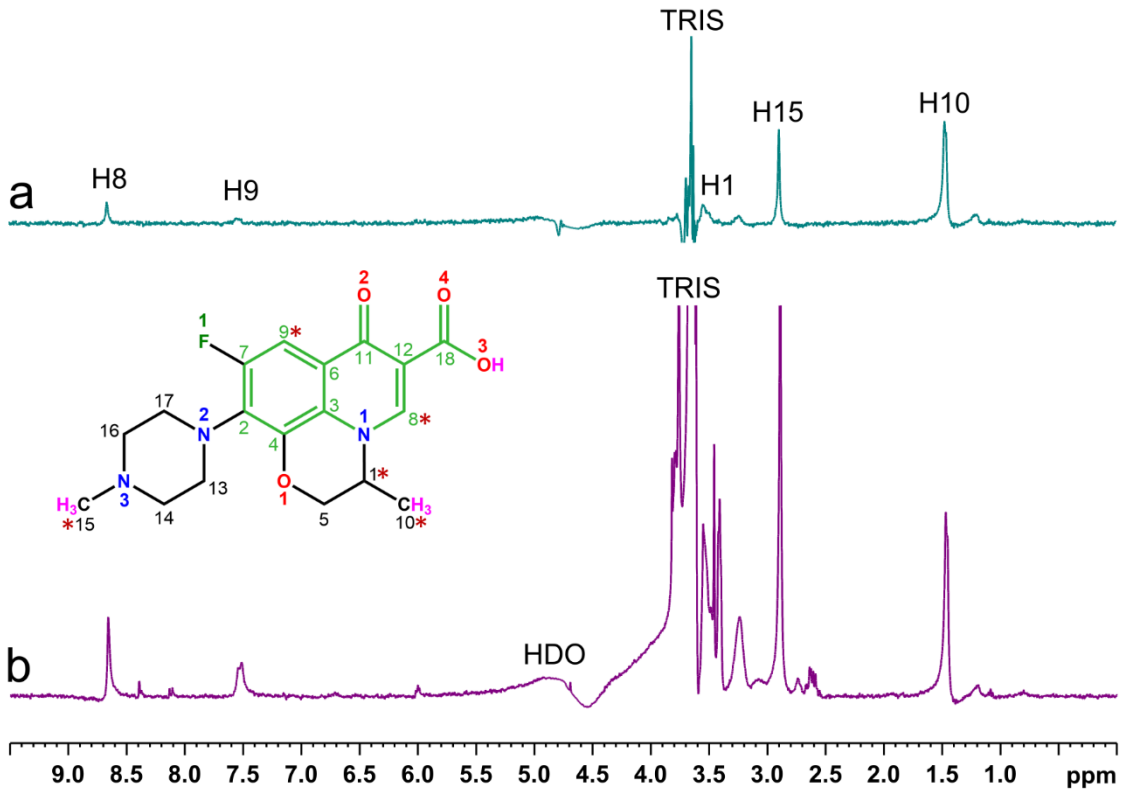

Fig.S12. (a) STD spectrum of OFX in presence of actin; (b) Reference spectrum (off resonance) of OFX in presence of actin. The compound was added to 2mM final concentration to result in a 50-fold excess of the ligand.

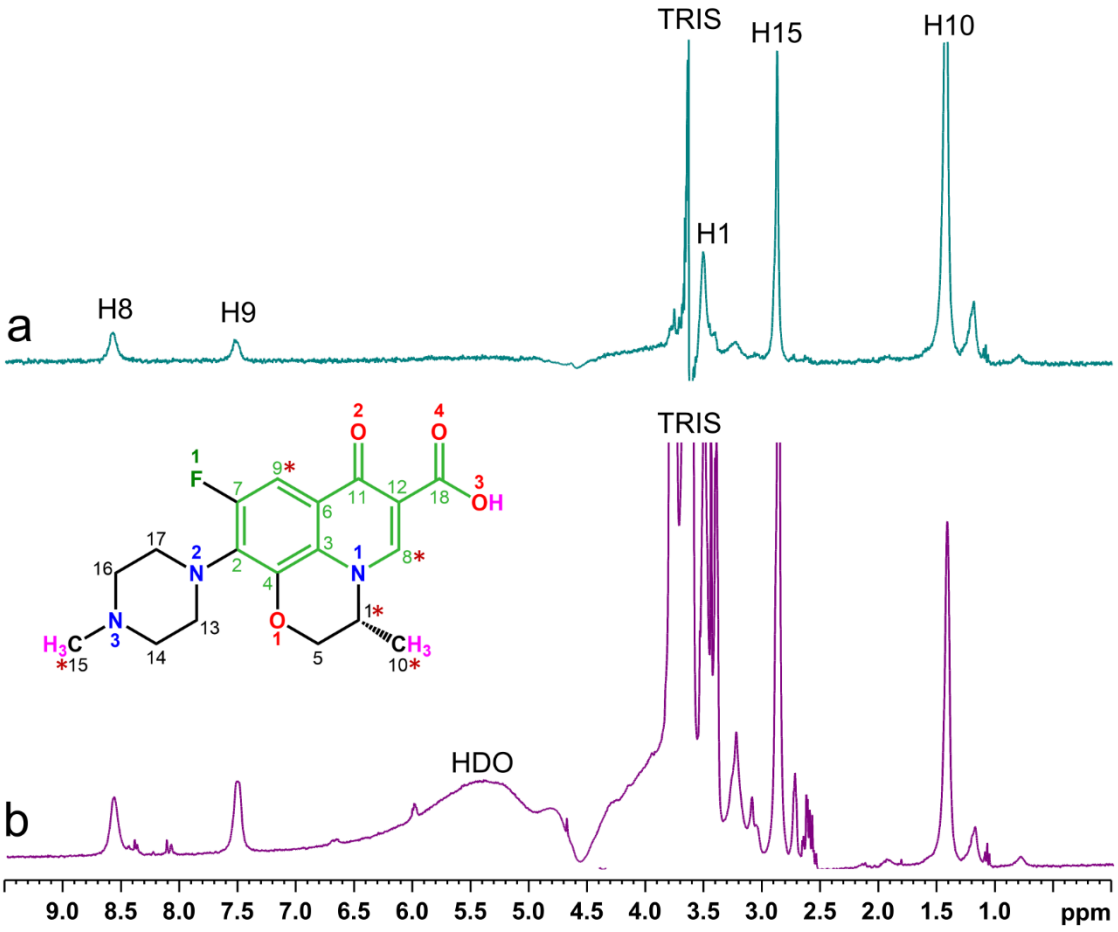

Fig.S13. (a) STD spectrum of LFX in presence of actin; (b) Reference spectrum (off resonance) of LFX in presence of actin. The compound was added to 2mM final concentration to result in a 50-fold excess of the ligand.

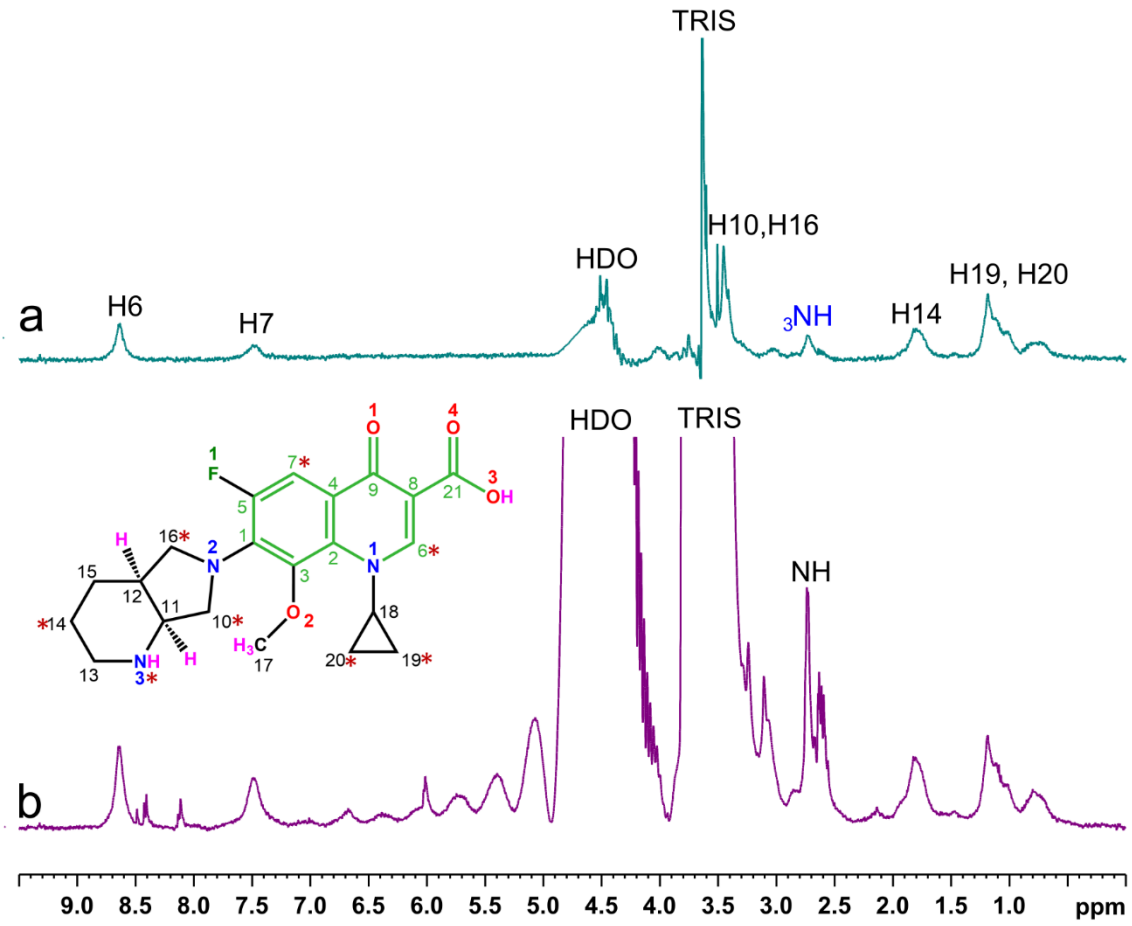

Fig.S14. (a) STD spectrum of MFX in presence of actin; (b) Reference spectrum (off resonance) of MFX in presence of actin. The compound was added to 2mM final concentration to result in a 50-fold excess of the ligand.

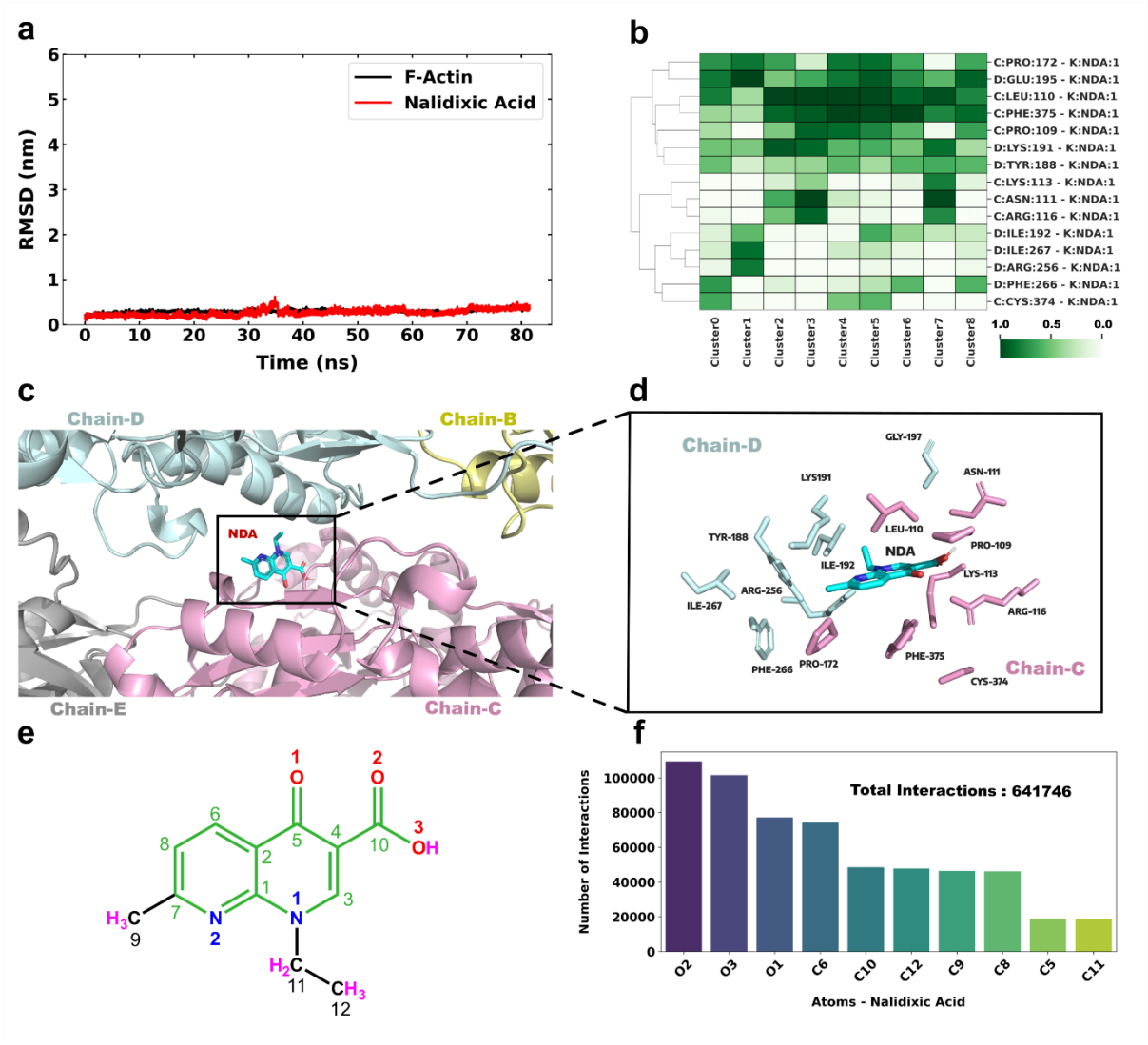

Fig.S15. Interaction dynamics of F-actin with NDA. (a) RMSD plot of the pentameric F-actin–NDA complex with F-actin in black and NDA in red; (b) Heatmap representation of frequently interacting amino acids across all the trajectories; (c) NDA, shown as sticks in cyan, bound at the interface of the pentameric F-actin represented as cartoon with Chain A (light blue), Chain B (yellow), and Chain C (pink); (d) Amino acid residues of F-actin (shown as sticks) interacting with NDA as seen in the heatmap; (e) 2D annotated structure of NDA; (f) Top 10 frequently interacting atoms of NDA (all trajectories) with F-actin.

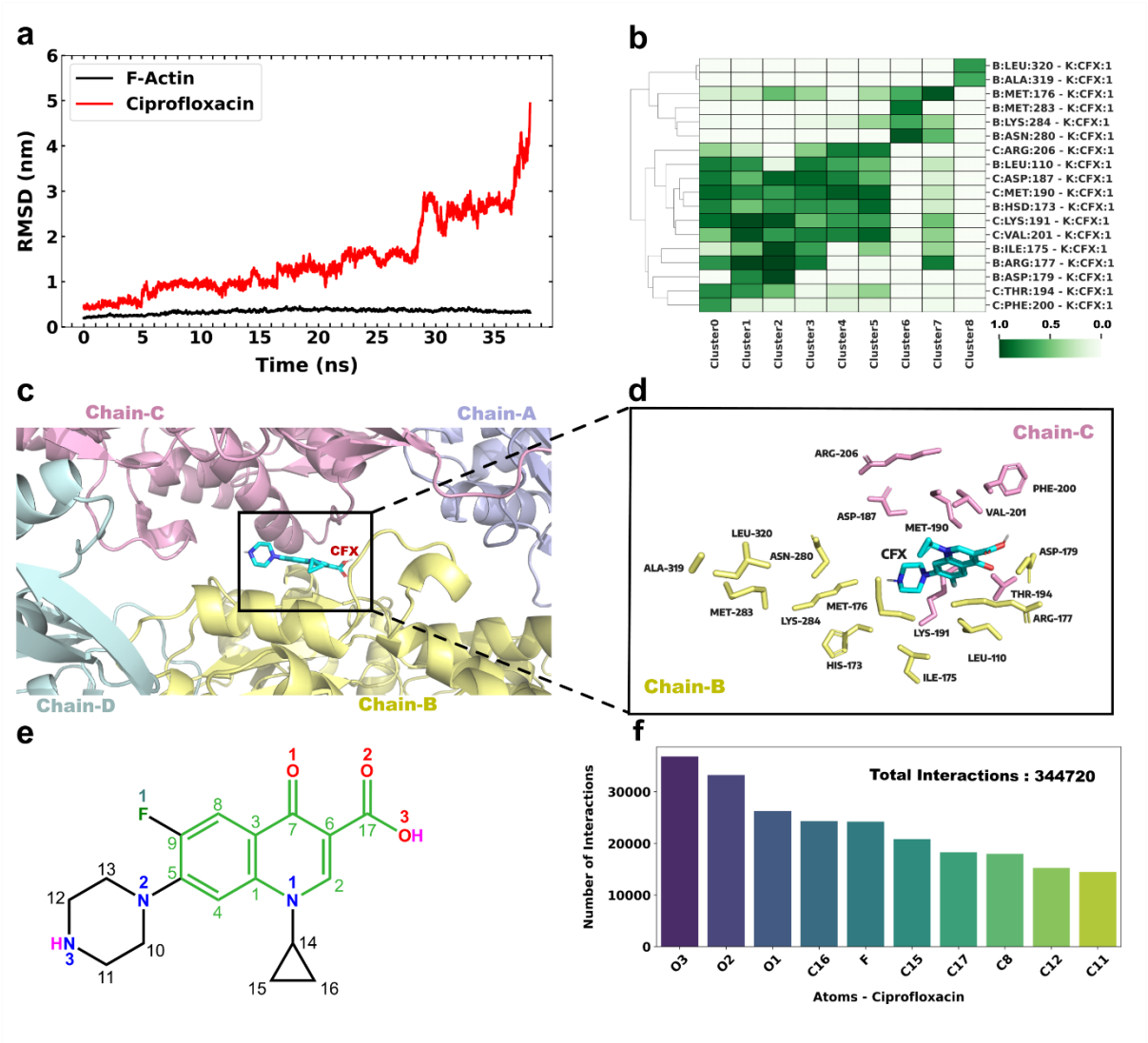

Fig.S16. Interaction dynamics of F-actin with CFX. (a) RMSD plot of the pentameric F-actin–CFX complex with F-actin in black and CFX in red; (b) Heatmap representation of frequently interacting amino acids across all the trajectories; (c) CFX, shown as sticks in cyan, bound at the interface of the pentameric F-actin represented as cartoon with Chain A (light blue), Chain B (yellow), and Chain C (pink); (d) Amino acid residues of F-actin (shown as sticks) interacting with CFX as seen in the heatmap; (e) 2D annotated structure of CFX; (f) Top 10 frequently interacting atoms of CFX (all trajectories) with F-actin.

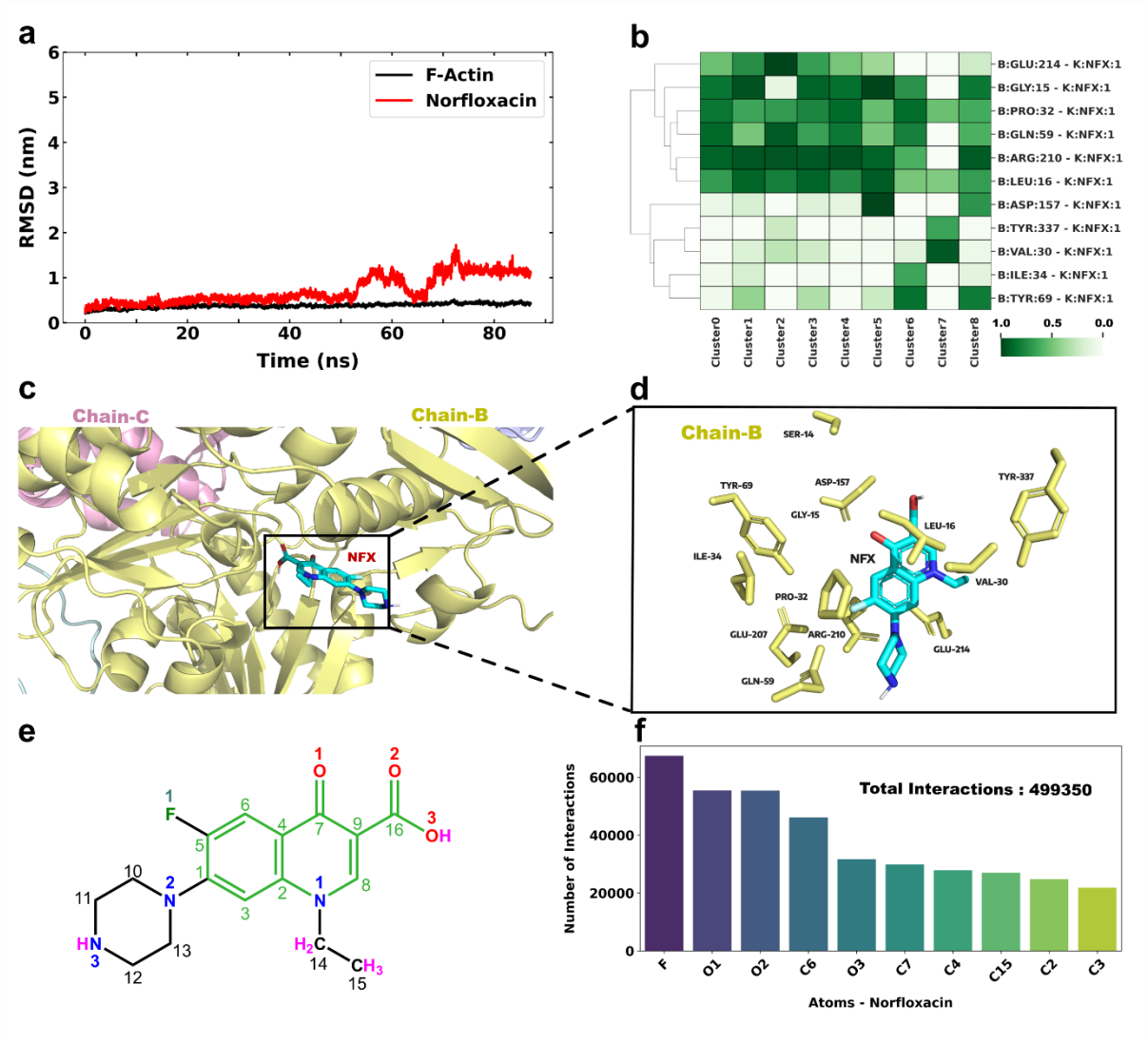

Fig.S17. Interaction dynamics of F-actin with NFX. (a) RMSD plot of the pentameric F-actin–NFX complex with F-actin in black and NFX in red; (b) Heatmap representation of frequently interacting amino acids across all the trajectories; (c) NFX, shown as sticks in cyan, bound at the interface of the pentameric F-actin represented as cartoon with Chain A (light blue), Chain B (yellow), and Chain C (pink); (d) Amino acid residues of F-actin (shown as sticks) interacting with NFX as seen in the heatmap; (e) 2D annotated structure of NFX; (f) Top 10 frequently interacting atoms of NFX (all trajectories) with F-actin.

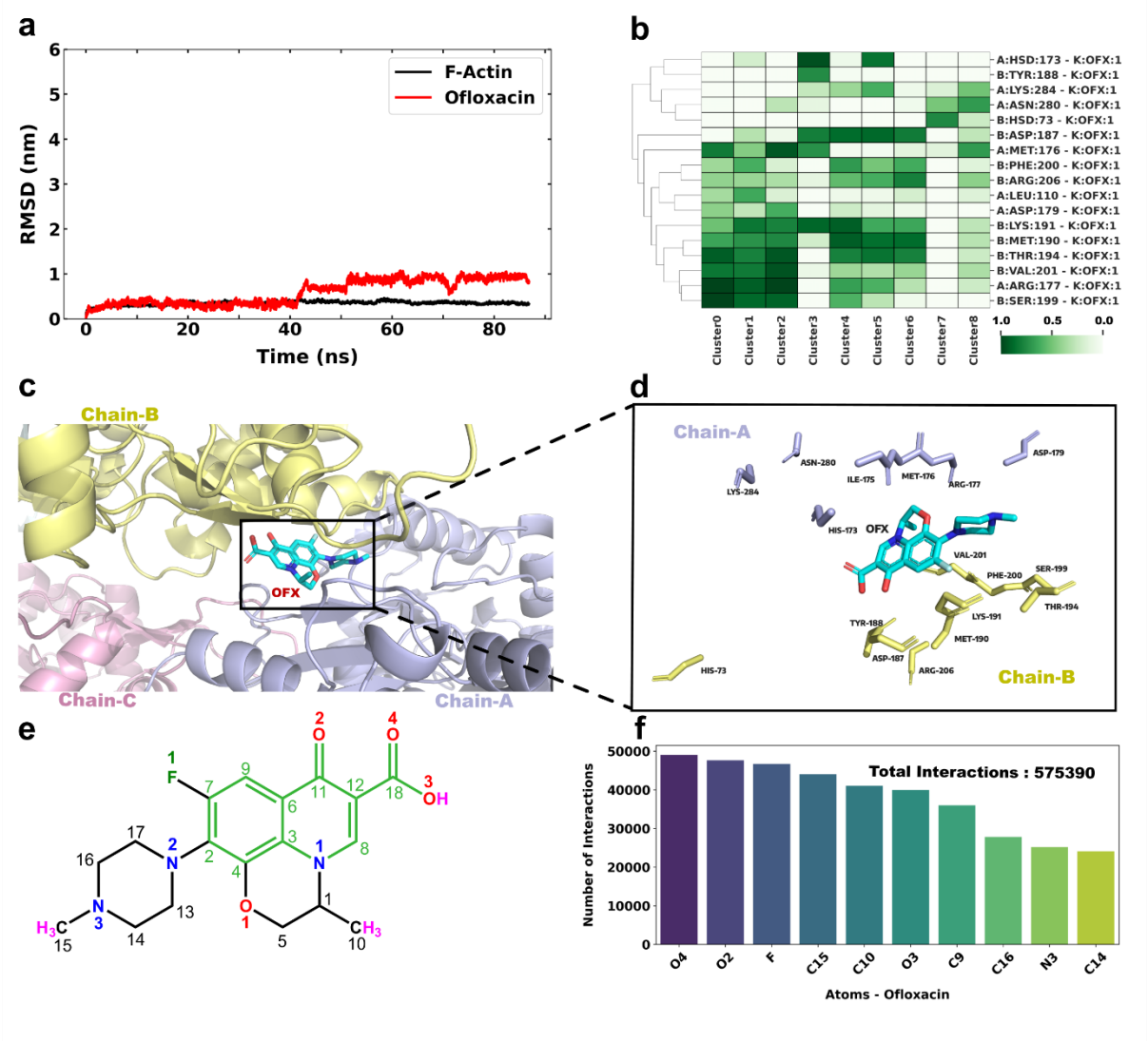

Fig.S18. Interaction dynamics of F-actin with OFX. (a) RMSD plot of the pentameric F-actin–OFX complex with F-actin in black and OFX in red; (b) Heatmap representation of frequently interacting amino acids across all the trajectories; (c) OFX, shown as sticks in cyan, bound at the interface of the pentameric F-actin represented as cartoon with Chain A (light blue), Chain B (yellow), and Chain C (pink); (d) Amino acid residues of F-actin (shown as sticks) interacting with OFX as seen in the heatmap; (e) 2D annotated structure of OFX; (f) Top 10 frequently interacting atoms of OFX (all trajectories) with F-actin.

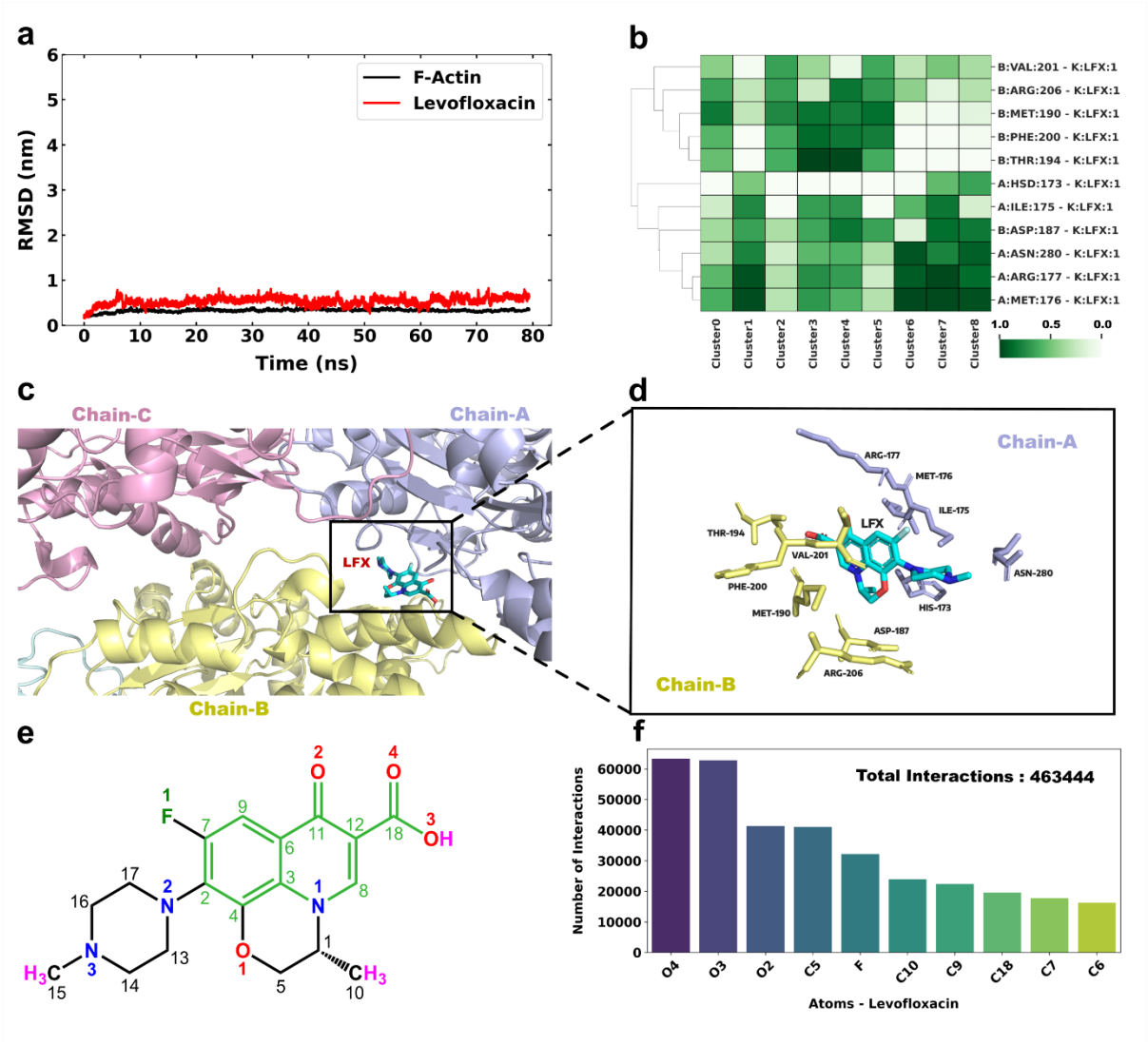

Fig.S19. Interaction dynamics of F-actin with LFX. (a) RMSD plot of the pentameric F-actin–LFX complex with F-actin in black and LFX in red; (b) Heatmap representation of frequently interacting amino acids across all the trajectories; (c) LFX, shown as sticks in cyan, bound at the interface of the pentameric F-actin represented as cartoon with Chain A (light blue), Chain B (yellow), and Chain C (pink); (d) Amino acid residues of F-actin (shown as sticks) interacting with LFX as seen in the heatmap; (e) 2D annotated structure of LFX; (f) Top 10 frequently interacting atoms of LFX (all trajectories) with F-actin.

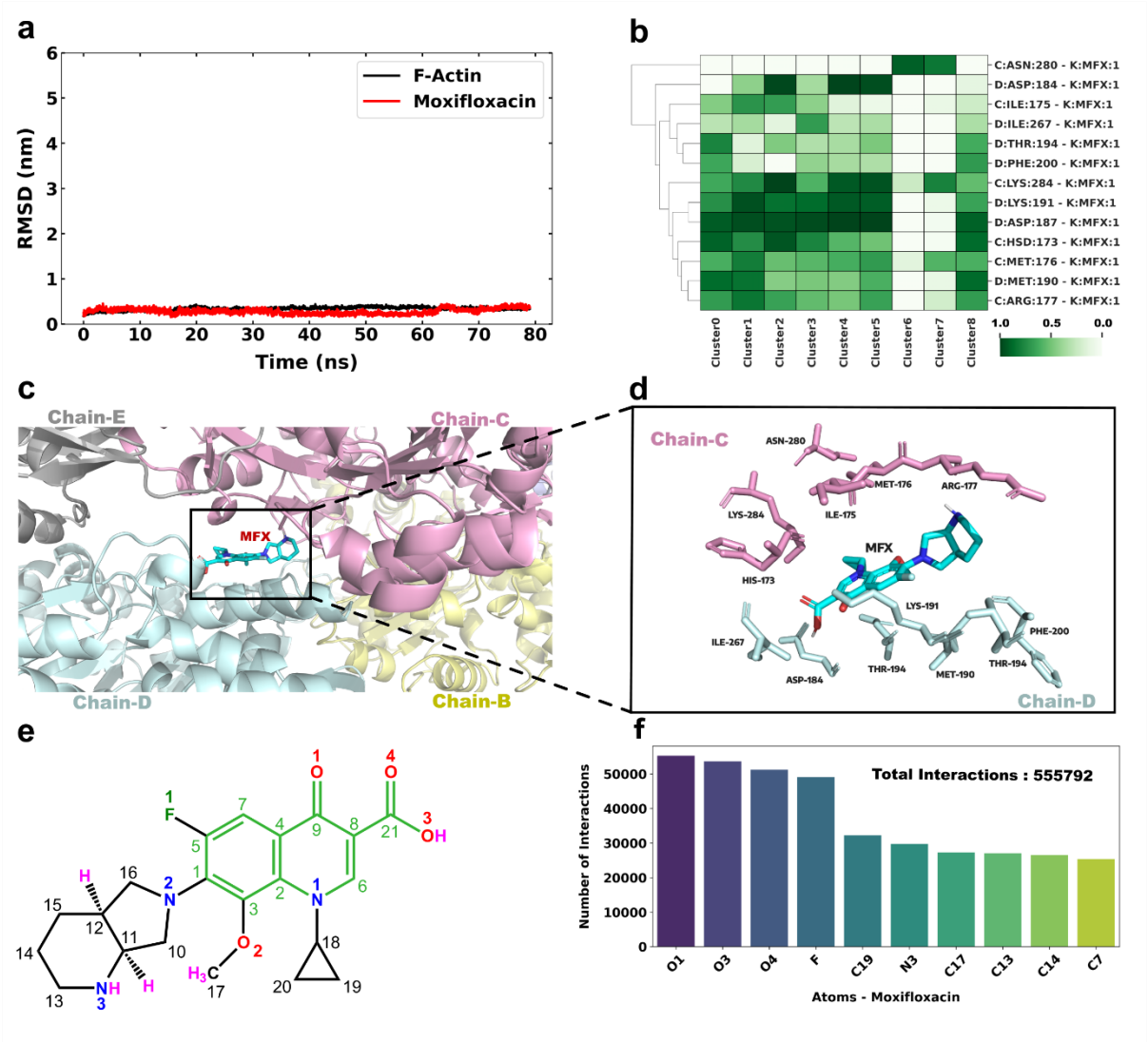

Fig.S20. Interaction dynamics of F-actin with MFX. (a) RMSD plot of the pentameric F-actin–MFX complex with F-actin in black and MFX in red; (b) Heatmap representation of frequently interacting amino acids across all the trajectories; (c) MFX, shown as sticks in cyan, bound at the interface of the pentameric F-actin represented as cartoon with Chain A (light blue), Chain B (yellow), and Chain C (pink); (d) Amino acid residues of F-actin (shown as sticks) interacting with MFX as seen in the heatmap; (e) 2D annotated structure of MFX; (f) Top 10 frequently interacting atoms of MFX (all trajectories) with F-actin.

Supplementary Tables

Table S1: Secondary structure content of F-actin upon treatment with fluoroquinolones (FQs) at various molar ratios.

		Secondary structural content			
Drugs	**Molar Ratio**	**Helix (%)**	**Sheet (%)**	**Turns (%)**	**Others (%)**
3 µM F-actin (control)		30.00	23.50	12.20	34.30
NDA	1:5	31.80	20.90	12.60	34.70
	1:10	32.30	21.10	13.10	33.50
	1:30	34.10	21.10	12.10	32.40
CFX	1:5	27.50	23.20	13.20	36.10
	1:10	31.00	17.50	13.30	38.20
	1:30	27.10	27.00	12.40	33.50
NFX	1:5	33.00	21.30	12.60	33.10
	1:10	31.20	21.30	12.50	35.00
	1:30	32.20	19.30	13.50	35.00
OFX	1:5	29.10	22.80	13.00	35.10
	1:10	26.70	25.40	12.40	35.50
	1:30	25.40	26.50	12.40	35.70
LFX	1:5	28.10	24.70	12.50	34.70
	1:10	34.00	18.40	13.40	34.20
	1:30	22.40	28.30	12.80	36.50
SFX	1:5	28.50	19.50	13.90	38.10
	1:10	29.20	23.20	13.00	34.60
	1:30	29.60	22.20	12.90	35.30
MFX	1:5	31.60	20.20	12.50	35.70
	1:10	28.80	20.30	13.20	37.70
	1:30	30.60	19.60	13.80	36.00

Table S2: Molecular Docking analysis of FQs against F-actin

Sr. No.	**Compound Name**	**Binding Energy (kcal.mol^{-1})**
1	NDA	-6.5
2	CFX	-7.6
3	NFX	-7.6
4	OFX	-7.7
5	LFX	-7.4
6	SFX	-7.8
7	MFX	-7.7