Science Advances
AAAS

Supplementary Materials for

Pf bacteriophages hinder sputum antibiotic diffusion via electrostatic binding

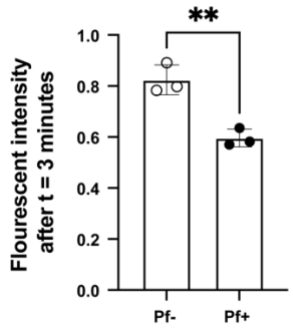
Qingquan Chen *et al.*

Corresponding author: Andy Spakowitz, Paul L. Bollyky, pbollyky@stanford.edu

Sci. Adv. **10**, eadl5576 (2024)
DOI: 10.1126/sciadv.adl5576

This PDF file includes:

Figure S1. Fluorescence intensity after max recovery observation time of 3 minutes for Cy5-TOB in sputum with Pf and without Pf spiked in



The fluorescence intensity of Cy5-TOB after the max observation time of 3 minutes is plotted for the patient sputum samples that were and were not spiked with Pf.

Figure S2. FRAP recovery curves for Cy5-TOB and Cy5-CIP in patient sputum with and without Pf. Fluorescence intensity recovery curves for **(A)** Cy5-TOB and **(B)** Cy5-CIP in patient sputum with and without Pf.

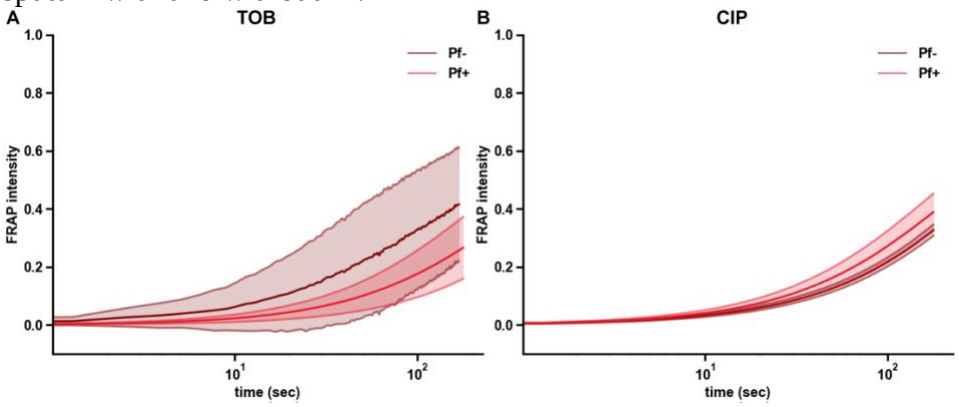


Figure S3. FRAP recovery curves for Cy5-TOB and Cy5-CIP in artificial sputum with and without Pf. Fluorescence intensity recovery curves for **(A)** Cy5-TOB and **(B)** Cy5-CIP in patient sputum with and without Pf. The effective diffusion coefficients (tau^{-1}) of **(C)** Cy5-TOB in PBS (n=12), Pf (n=10), artificial sputum (n=8), and artificial sputum with Pf (n=6) and **(D)** Cy5-CIP in PBS (n=9), PF (n=9), artificial sputum (n=6), and artificial sputum with Pf (n=6) are shown.

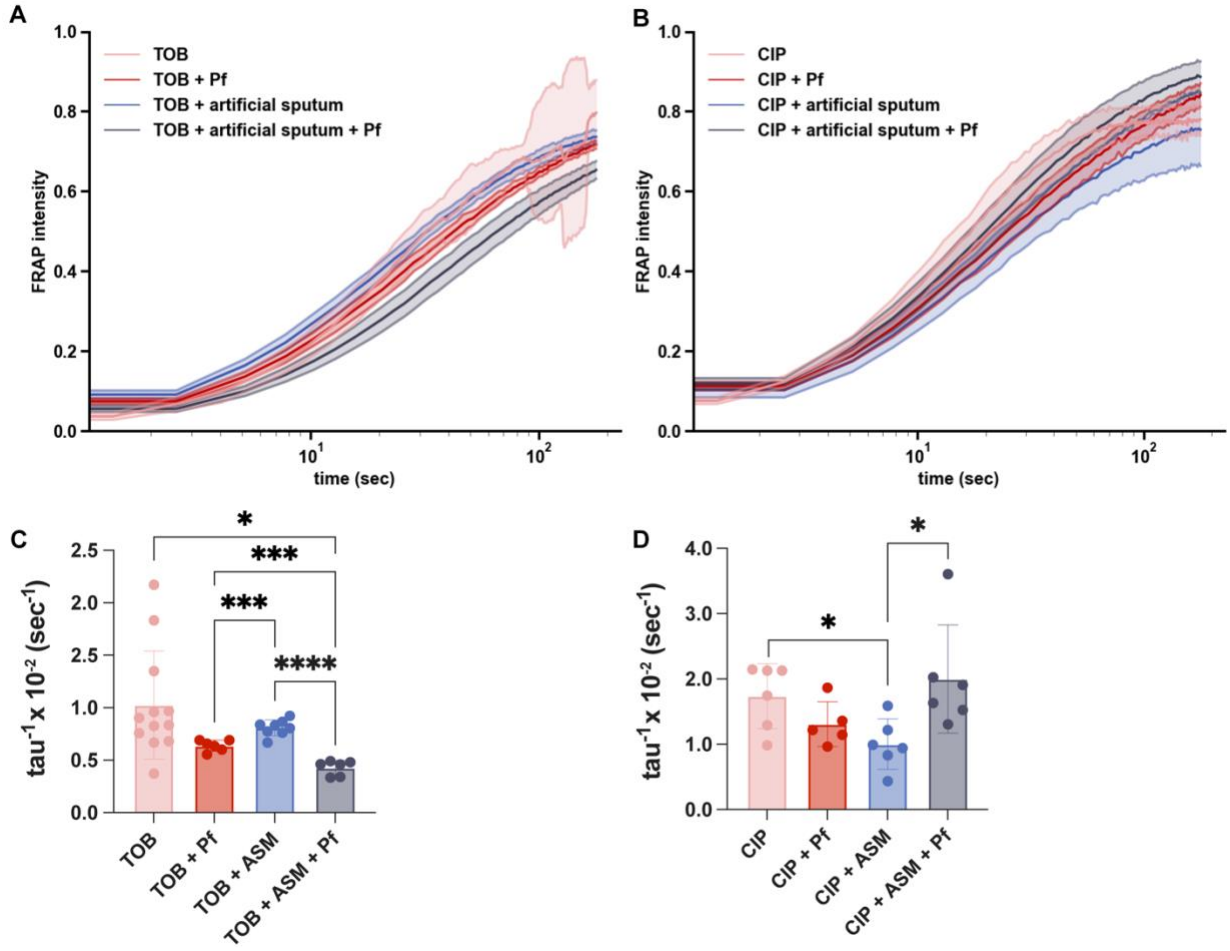
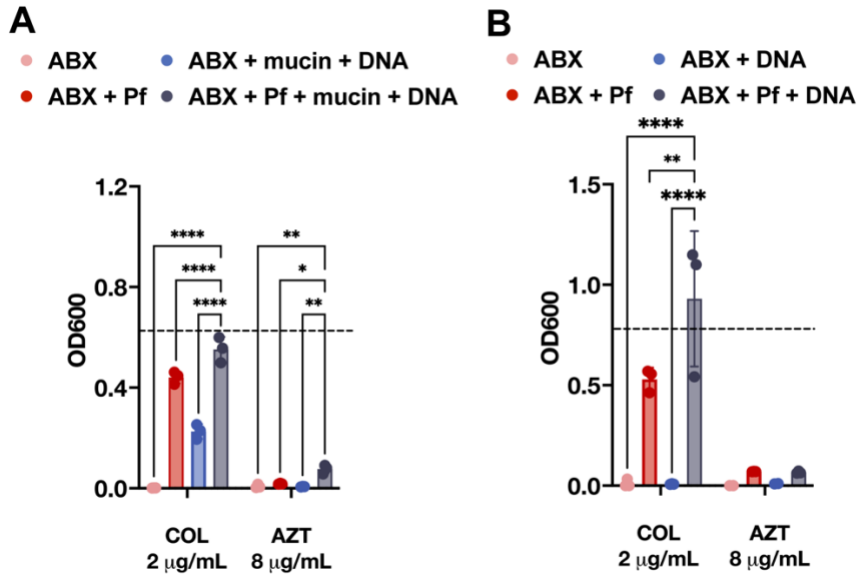


Figure S4. Pf and sputum polymers affect the efficacy of colistin and aztreonam.



To assess the impact of filamentous bacteriophage (Pf) and lung polymers on antibiotic efficacy, we measured antibiotic killing of *Pseudomonas aeruginosa* (*Pa*) (n=3) for COL and AZT in Pf and DNA conditions with mucin present **(A)** and without mucin present **(B)**. antibiotics = ABX; colistin = COL; aztreonam = AZT.

Figure S5. FRAP intensity recovery curves for tobramycin and ciprofloxacin in sputum polymers and mucin. The fluorescence intensity as a function of time as measured after photobleaching is shown for **(A)** Cy5-TOB and **(B)** Cy5-CIP in the presence of combinations of mucin, DNA, and Pf.

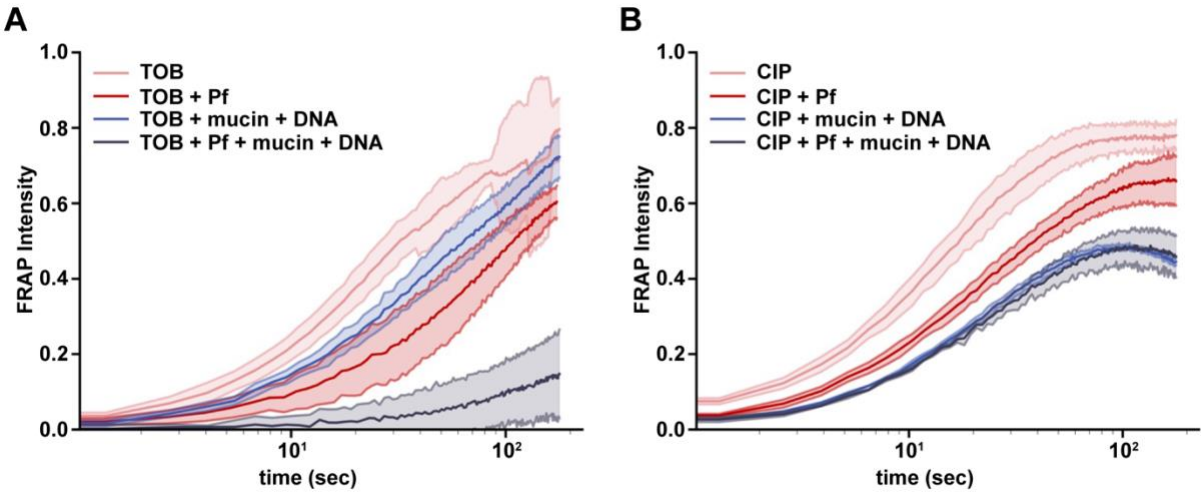
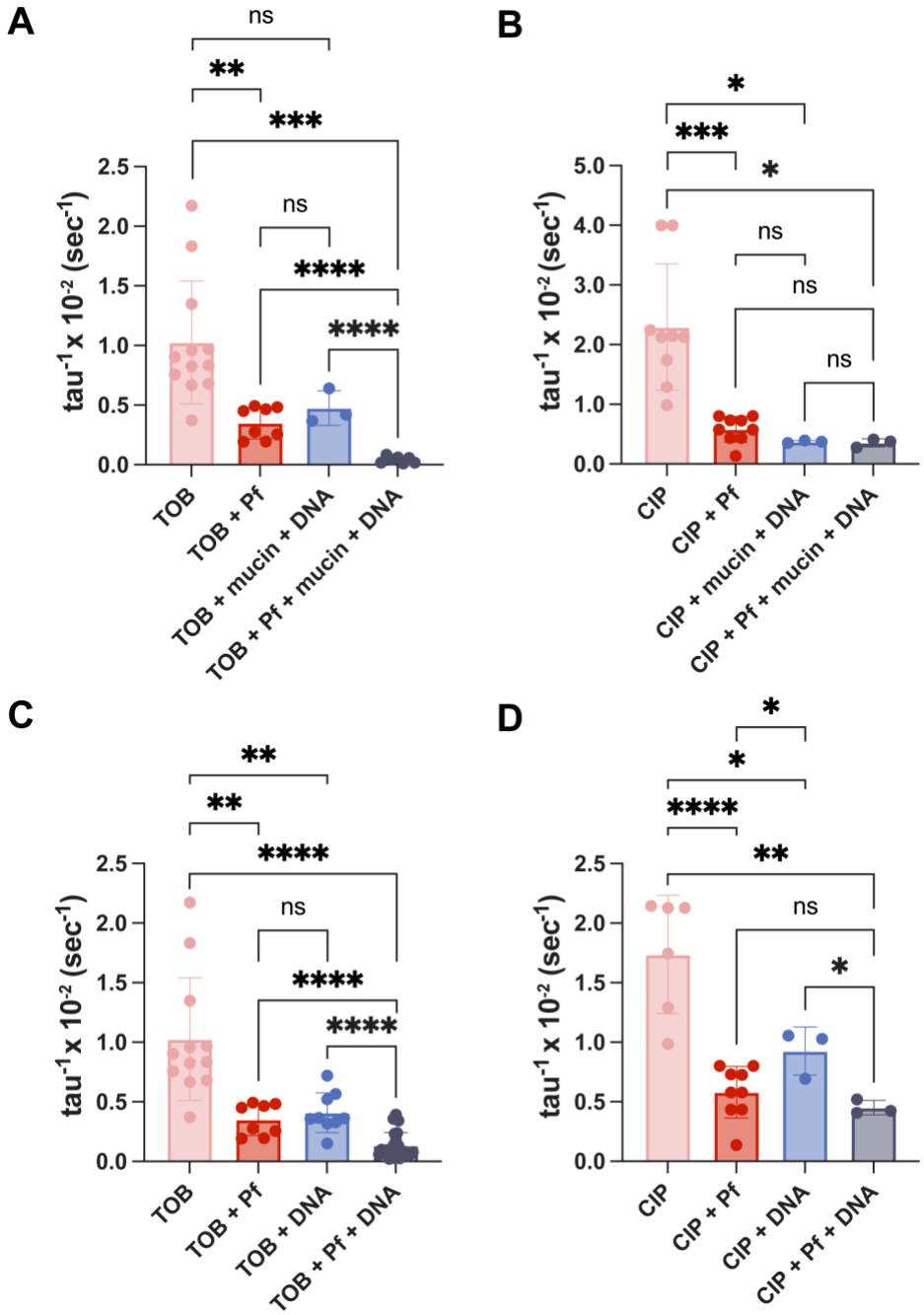


Figure S6. Additional pairwise statistical comparisons for effective diffusion constants derived from FRAP experiments.



The effective diffusion rate, tau^{-1}, is shown for **(A)** TOB and **(B)** CIP in simple sputum with all comparison statistics shown (same data as **Fig. 2B, C**). The effective diffusion is also shown for **(C)** TOB and **(D)** and CIP in DNA solutions with all comparison statistics shown (same data as **Fig. 4E, F**).

Figure S7. Isothermal titration calorimetry of antibiotics into Pf and mucin. The **(A)** binding affinities and **(B)** entropy changes for Cy5-CIP and Cy5-TOB in the presence of Pf and Pf with mucin.

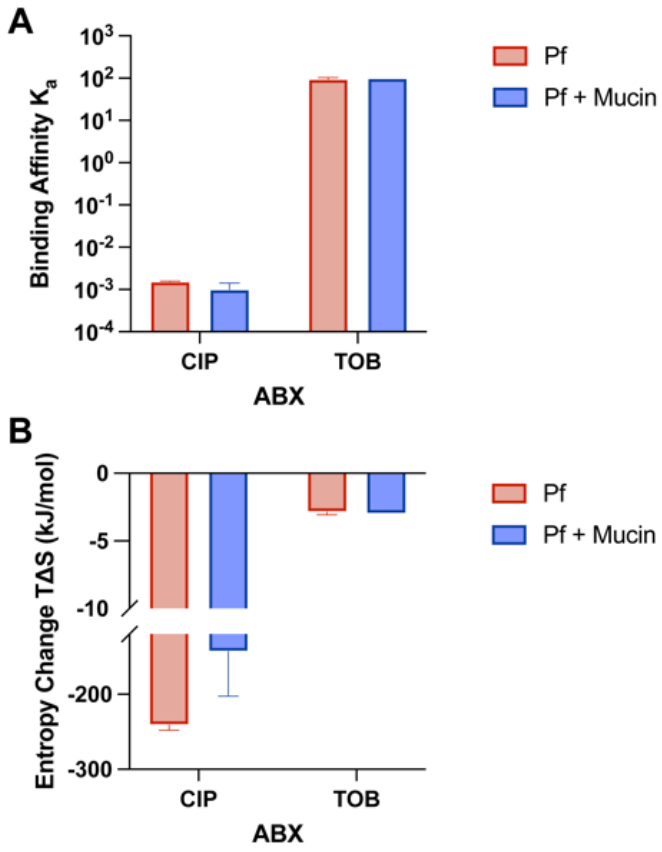
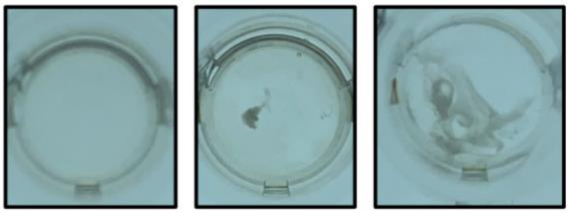


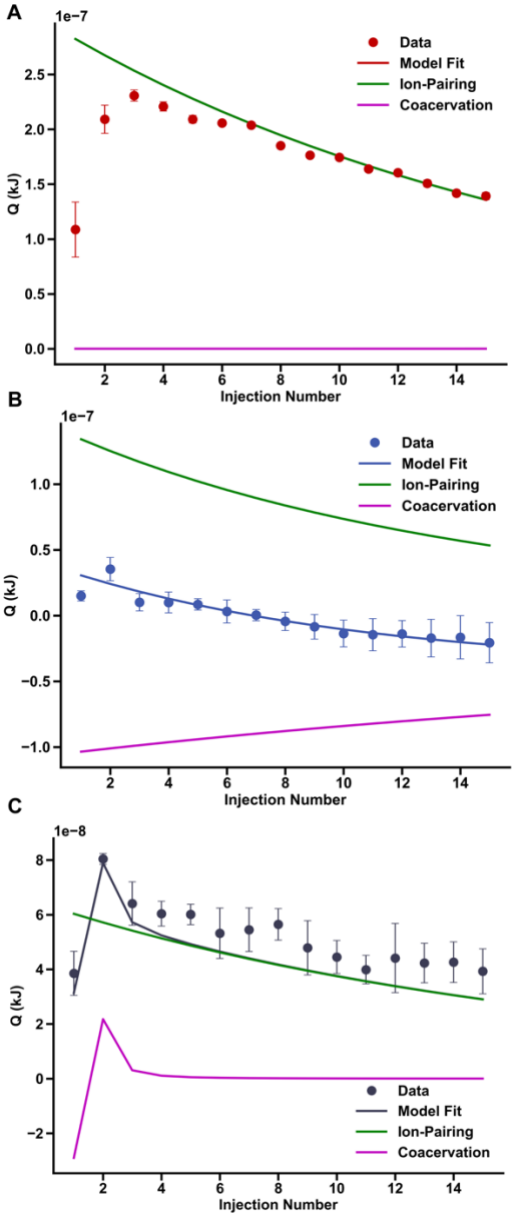
Figure S8. Phase change in the presence of tobramycin, Pf phages, and DNA.



Pf	+	-	+
DNA	-	+	+
TOB	+	+	+

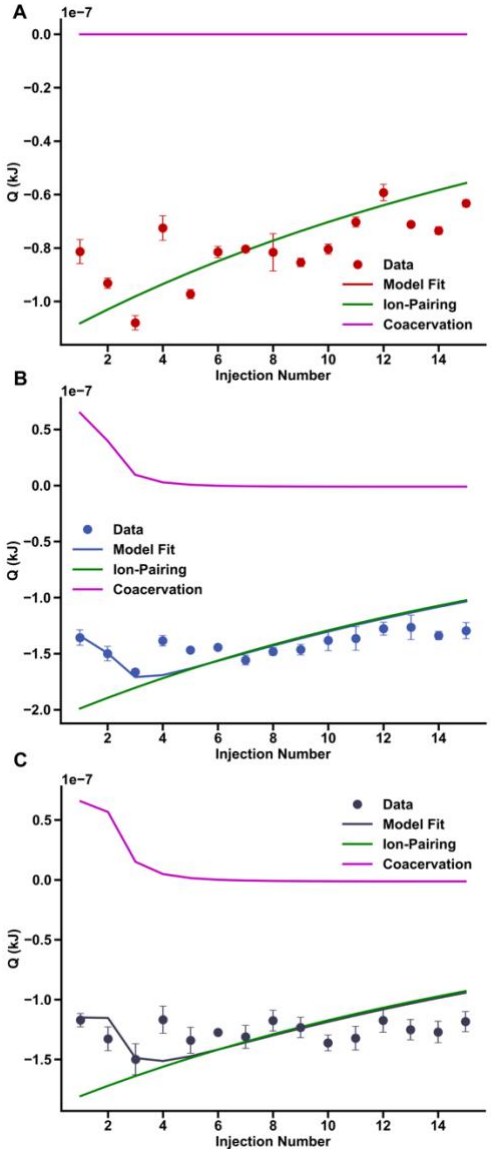
Pf phages (10^{11} pfu/ml), DNA (4 mg/mL), and tobramycin (1 mg/mL) were mixed as indicated above.

Figure S9. Heat released per injection from ITC measurements of tobramycin with sputum components.



(A-C) Fitted isotherm for tobramycin in 10e11 pfu/mL Pf4, 4 mg/mL DNA, and 10e11 pfu/mL Pf4 with 4 mg/mL DNA, along with relative contributions from the coacervation and the ion pairing.

Figure S10. Heat released per injection from ITC measurements of ciprofloxacin with sputum components.



(A-C) Fitted isotherm for ciprofloxacin in 10e11 pfu/mL Pf4, 4 mg/mL DNA, and 10e11 pfu/mL Pf4 with 4 mg/mL DNA, along with relative contributions from the coacervation and the ion pairing.

Figure S11. FRAP recovery curves for Cy5-TOB and Cy5-CIP in conditions of PBS, Pf, DNA, and Pf + DNA. The fluorescence intensity from FRAP experiments of Cy5-TOB (A) and Cy5-CIP (B) in PBS (n = 12 for TOB, n = 9 for CIP), Pf (n = 9 for both TOB and CIP), DNA (n = 12 for TOB and n = 3 for CIP), and both Pf and DNA (n = 13 for TOB and n = 3 for CIP) are shown. Shaded regions indicate one standard deviation from the mean.

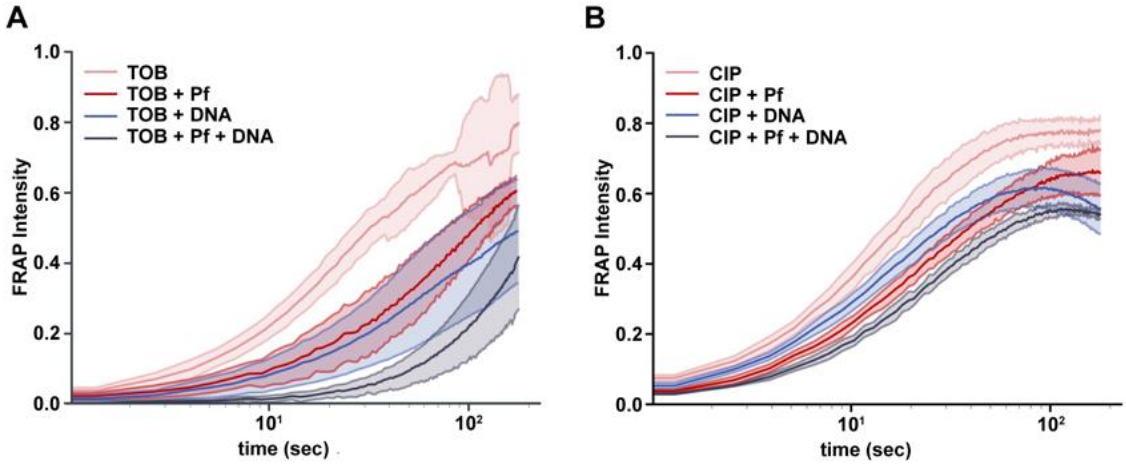


Figure S12. Effective diffusion rate, tau^{-1}, from FRAP recovery curves of Cy5-CIP. The effective diffusion rate from FRAP recovery curves of Cy5-CIP in PBS (n = 9), Pf (n = 9), DNA (n = 3), and both Pf and DNA (n = 3) are shown. (* indicates p≤0.05, ** indicates p≤0.01. Unpaired t-test.).

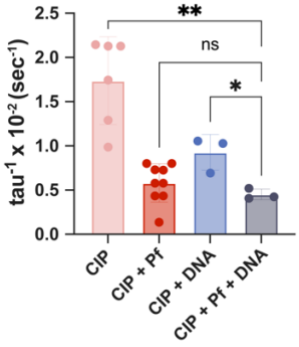


Figure S13. FRAP of 4kDa FITC-dextran reveals that diffusivity decreases for antibiotics are not mesh size-related (n=3). There is no statistical significance between the conditions.

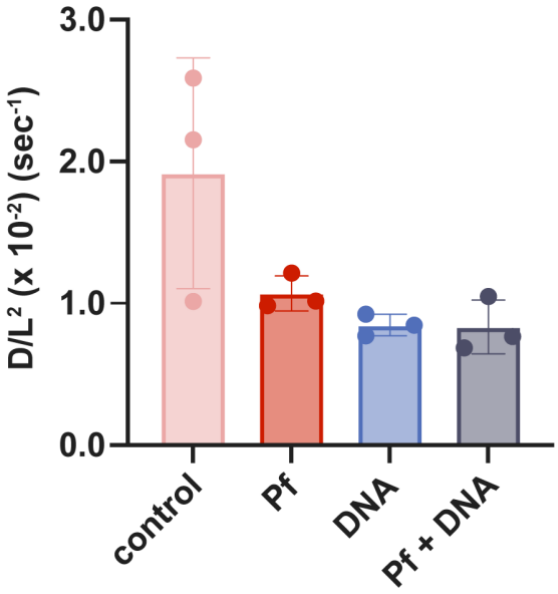
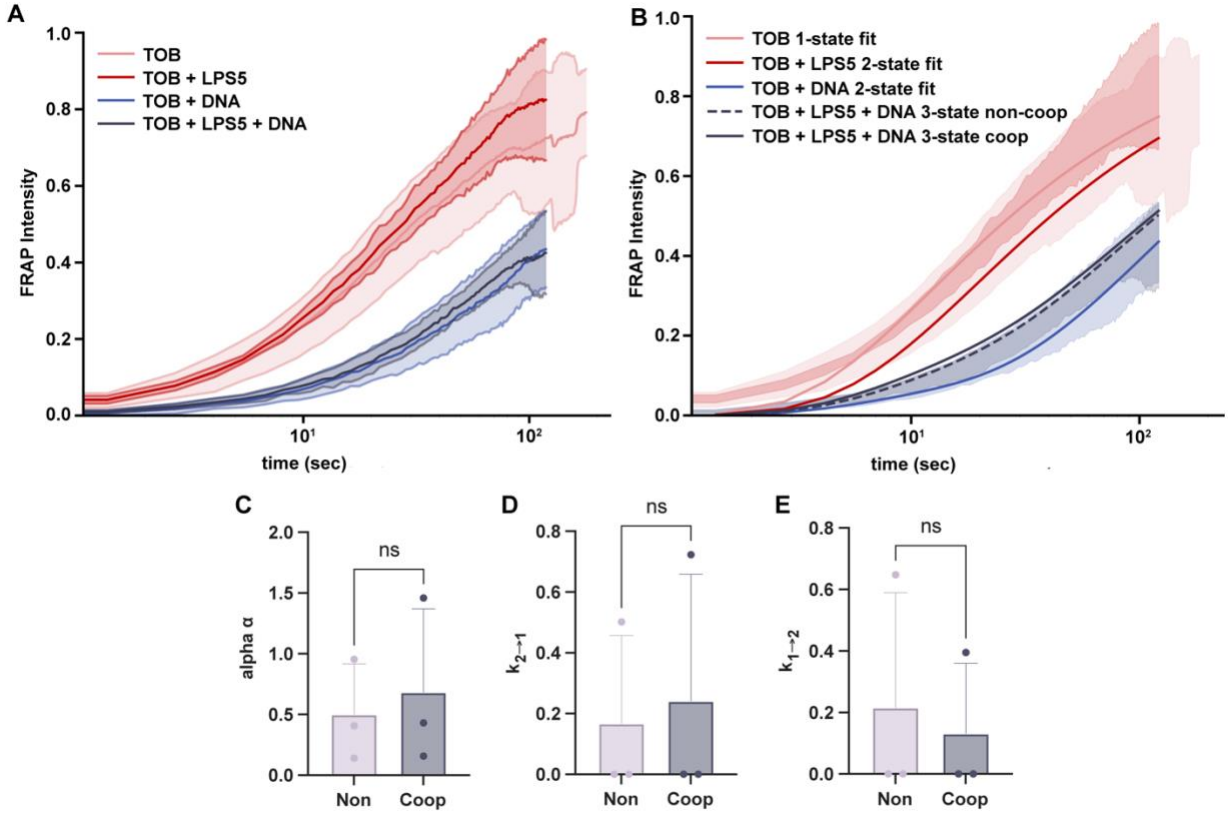


Figure S14. Tobramycin diffusion in non-liquid crystal-forming conditions was not affected.



(A) Average FRAP recovery curve with standard deviation for tobramycin in non-liquid crystal forming conditions of buffer, Lytic phage LPS5, DNA, and both LPS5 and DNA. The addition of LPS5 does not significantly decrease diffusion compared to just DNA. **(B)** Fitted FRAP recovery curves for each condition with a standard deviation of data in the background. The non-cooperative FRAP 3-state model shows very little difference from the cooperative FRAP 3-state model for tobramycin mixed with LPS5 and DNA. **(C-E)** LPS5 was mixed with DNA, where the phage does not form liquid crystals with polymer. Tobramycin diffusion was not slower in solutions of LPS5 and DNA.

Figure S15. 1-D line with Fluorescent Particle. A region of length L is shown with a fluorescent particle at position $x = x_0$.

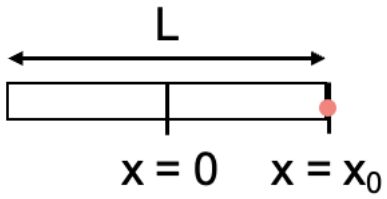
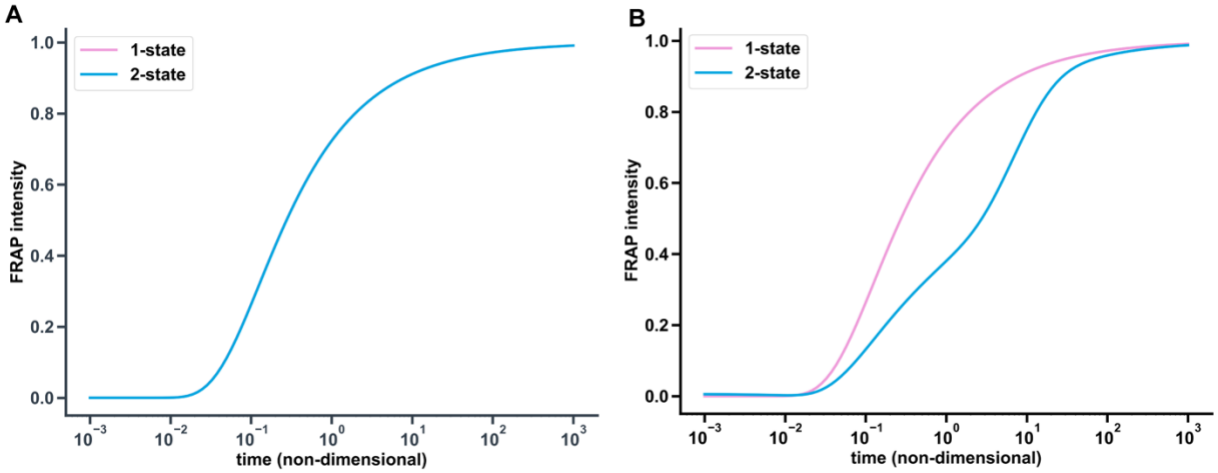


Figure S16. 2-State Model Theoretical Output.



(A) Setting the diffusion rate and rate constants equal for the two states in the 2-state model recovers the 1-state model FRAP curve. **(B)** When the bound state in the 2-state model experiences a lower diffusion rate, the model predicts a slower recovery curve, though eventually the curves recover to 1 with enough time.

Figure S17. Sensitivity analysis of the 2-state model. The fluorescence intensity curves are shown for a variety of 2-state model parameters. (**A**) shows the effect of varying the relative diffusion rate while keeping the binding rates constant. Next, the effect of varying the binding rate constant while keeping the unbinding rate constant at the same value is shown for low values (**B**) and high values (**C**) of the relative diffusion rate. Finally, the effect of varying the unbinding rate constant while keeping the unbinding rate constant at the same value is shown for low values (**D**) and high values (**E**) of the relative diffusion rate.

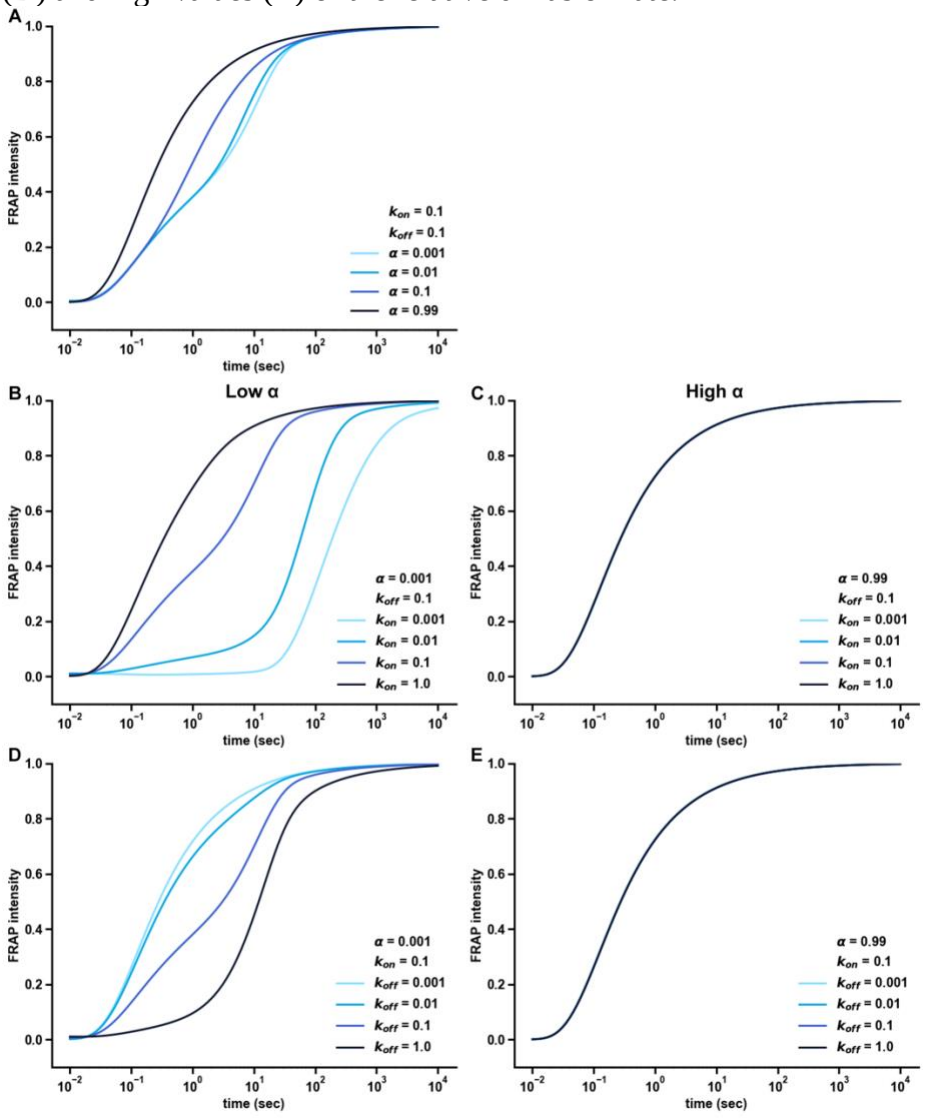
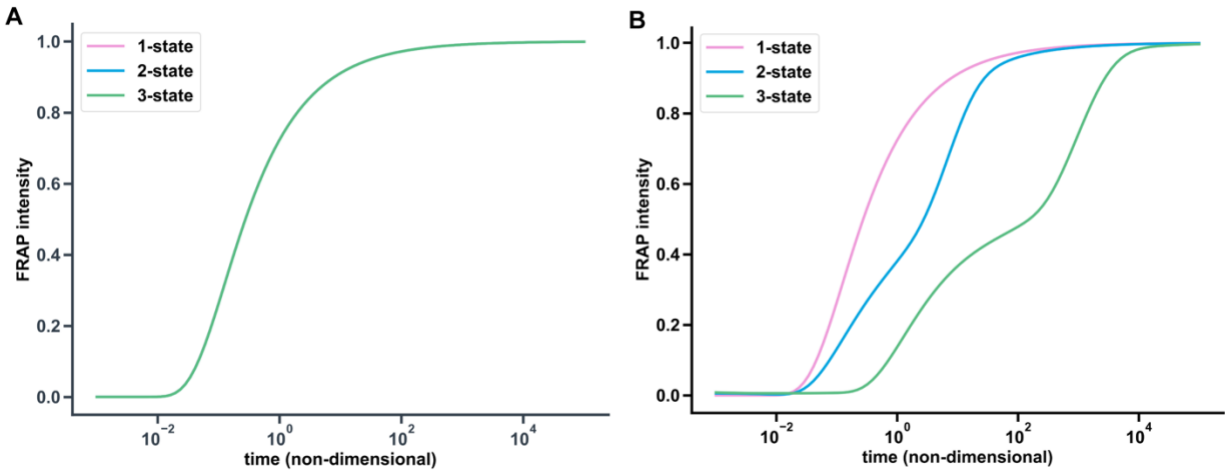


Figure S18. 3-State Model Theoretical Output.



(A) Setting the diffusion rate and rate constants equal for the three states in the 3-state model recovers the 1-state model FRAP curve. **(B)** When the bound states of the 3-state model experience lower diffusion rates, the model predicts a slower recovery curve than even in the 2-state model because of the additional state.

Figure S19. Sensitivity analysis of the 3-state model. The fluorescence intensity curves are shown for a variety of 3-state model parameters. The effect of varying the relative diffusion rate of state 1 while keeping all other parameters constant is shown for a low (**A**) and high (**B**) value of the other diffusion rate. The effect of varying the relative diffusion rate of state 2 while keeping all other parameters constant is shown for a low (**C**) and high (**D**) value of the other diffusion rate. The effect of varying the binding rate of state 2 while keeping all other parameters constant is shown for a low (**E**) and high (**F**) value of the diffusion rates. The effect of varying the unbinding rate of state 2 while keeping all other parameters constant is shown for a low (**G**) and high (**H**) value of the diffusion rates.

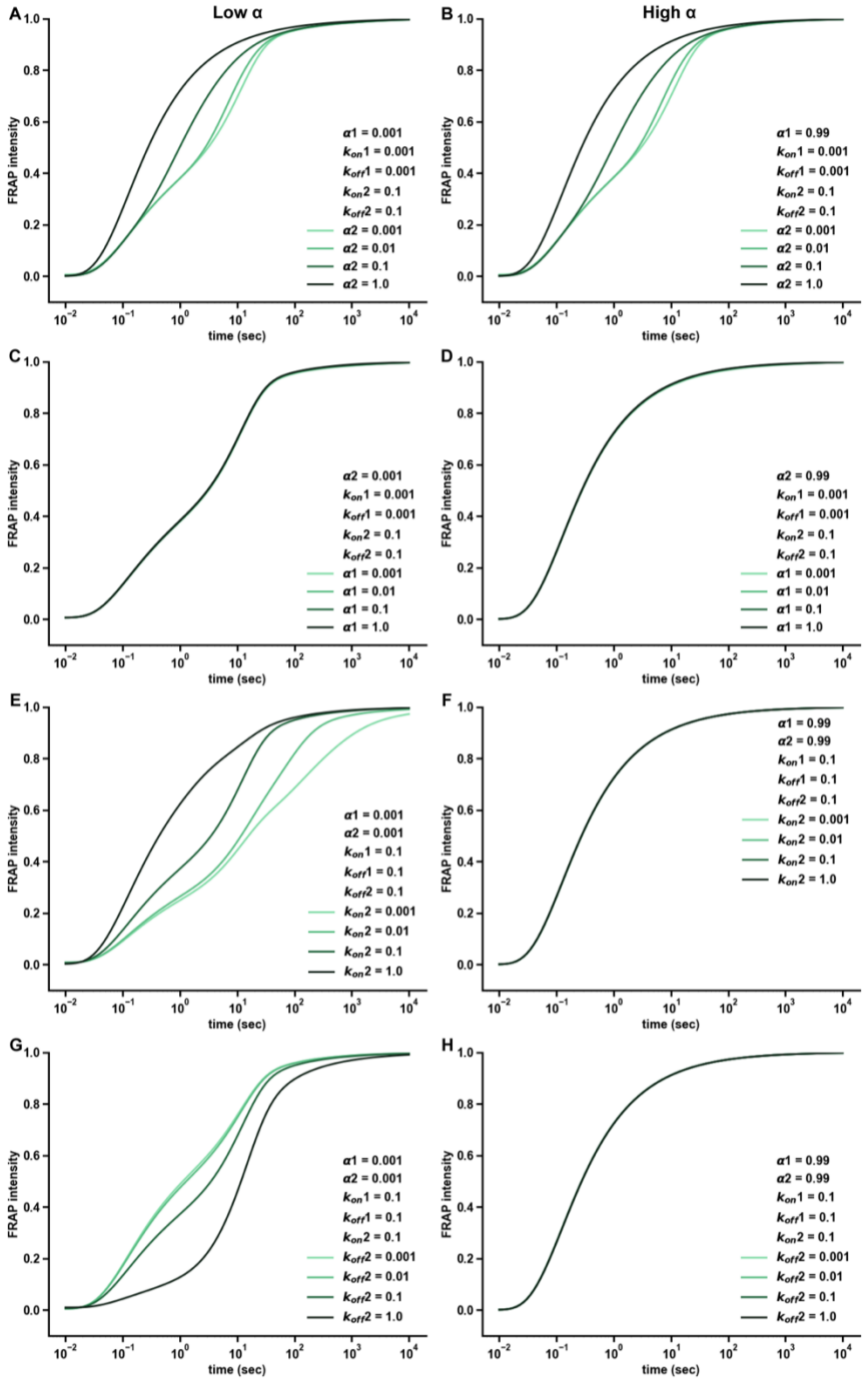
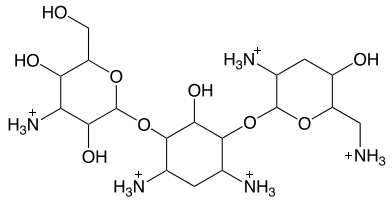
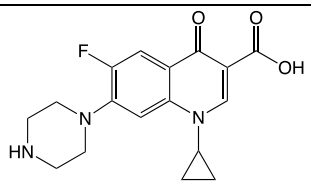
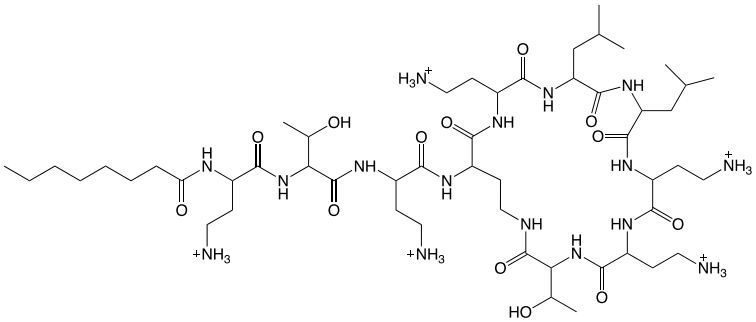
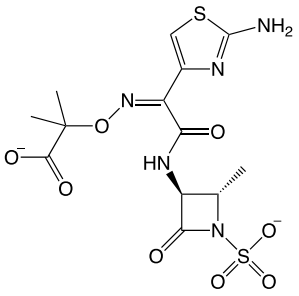


Table S1. Patients' information of sputum samples collected from.

Sputum Sample No.	Gender	Age (years)	*Pa* status	Pf status	On systemic antibiotics when sample collected
286	Male	31.1	None	Negative	No
334	Female	24.4	Chronic	Negative	No
421	Male	20.3	None	Negative	No

Table S2. The charge of different antibiotics under physiological pH=7

	Chemical Structure	Physiological charge
Tobramycin		**+5**
Ciprofloxacin		**0**
Colistin		**+5**
Aztreonam		**-2**