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Supplemental Information

Mode of Action of Antimicrobial Peptides on *E. coli* Spheroplasts

Yen Sun, Tzu-Lin Sun, and Huey W. Huang

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The mode of action of antimicrobial peptides on *E. coli* spheroplasts

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Department of Physics & Astronomy, Rice University, Houston Texas 77005

S1. Details of FRAP analysis

(1) Measurement of τ_e. During a dark period, the intracellular unbleached dye concentration $C(t)$ increased from the steady state value C_s to a higher value C_i when illumination resumed. Thereafter, $C(t)$ followed the kinetics:

$$C(t) - C_s = (C_i - C_s)e^{-t/\tau_e}$$

The decay constant τ_e was measured by curve fitting shown in Fig. S1.

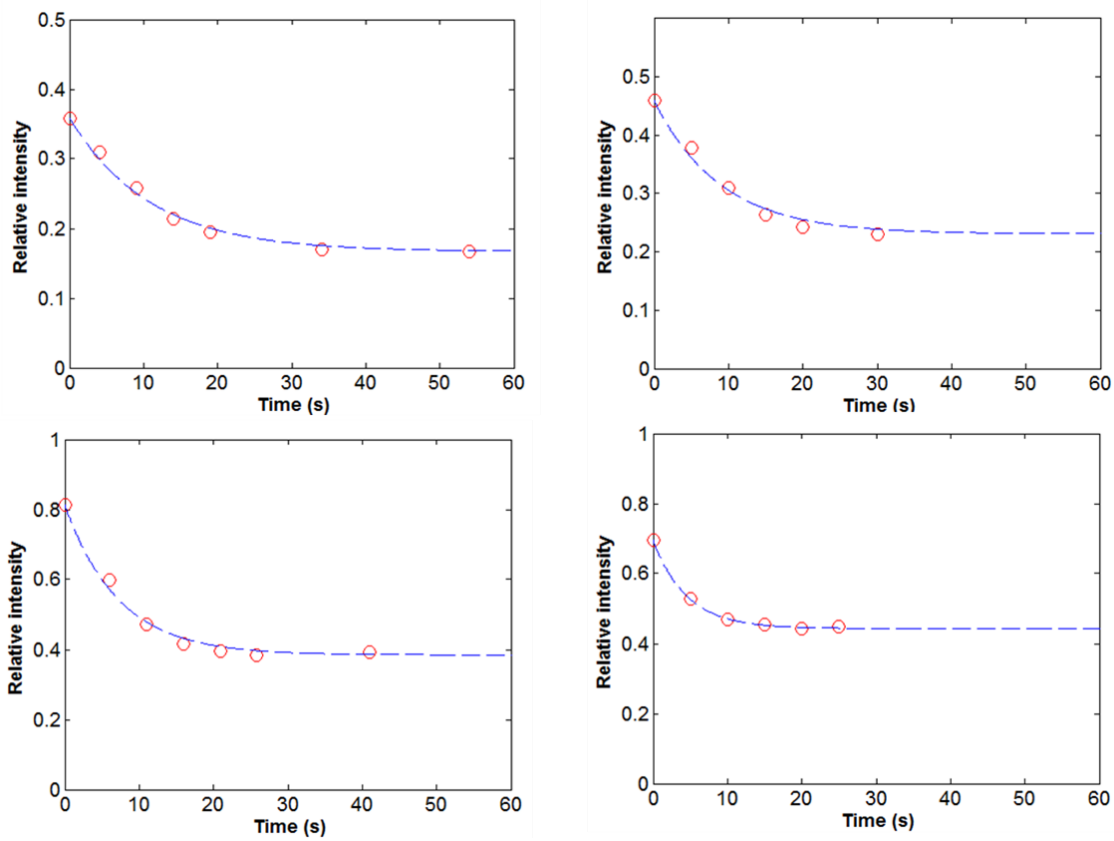

Fig. S1 Four examples of FRAP. Each was well fit by an exponential curve from which the value of τ_e was obtained.

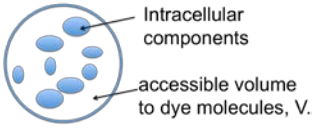

Fig. S2 Accessible volume in a cell

(2) We measured the intracellular fluorescence intensity, *F*, over the whole cell (Fig. S2). The intensity is proportional to the total number of unbleached fluorescence molecules within the measured volume. If the dye concentration *C* in the accessible volume *V* were the same as the extracellular concentration C_{out} which produced the extracellular fluorescence intensity F_{out}, the result would be

$$\frac{F}{F_{out}} = \frac{CV}{C_{out}V_{cell}} = \frac{V}{V_{cell}} \equiv x$$

Therefore, in all conditions, the correct ratio of the intracellular concentration *C* to the extracellular concentration C_{out} is obtained by the fluorescence measurement using the relation:

$$\frac{C}{C_{out}} = \frac{F}{F_{out} \cdot x}$$

Thus from $j_c = C_s V / C_{out}\tau_e$, we obtain $j_c = (F_s/F_{out})\, V_{cell}/\tau_e$.

(3) There is a second way of obtaining the value of j_c. During a dark period, there is no photobleaching, the kinetic equation for *C(t)* becomes

$$\frac{dC(t)}{dt} = -\frac{j_c}{V}C(t) + \frac{j_c}{V}C_{out}$$

During the steady state, j_c is constant, the equation can be integrated over the dark period: $C_{out} - C_i = (C_{out} - C_s)e^{-t1/\tau_j}$, where t1 is the duration of the dark period, $\frac{1}{\tau_j} = \frac{j_c}{V}$, C_s and C_i are the initial and final value of *C(t)*. Thus we can evaluate τ_j by:

$$1 - \frac{F_i}{F_{out} \cdot x} = \left(1 - \frac{F_s}{F_{out} \cdot x}\right) e^{-t1/\tau_j}$$

Then j_c is given by

$$j_c = \frac{V_{cell} \cdot x}{\tau_j}$$

The number of the hypothetical Berg-Purcell pores, N, was obtained by the formula $j_c = 4aDN$. The results obtained by two methods are compared in Fig. S3.

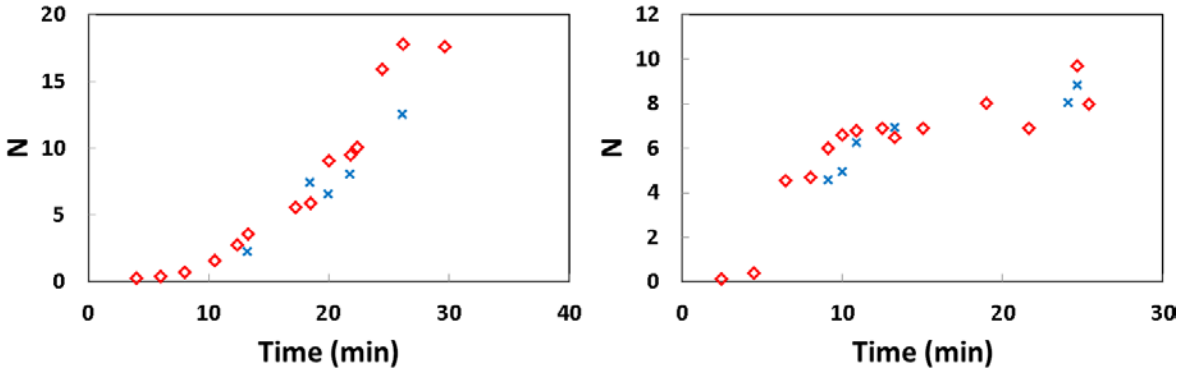

Fig. S3 The number of the hypothetical Berg-Purcell pores obtained from the two experiments shown in Fig. 2 by the first method (shown in red) and by the second method (shown in blue). The two methods yielded results close to each other.