Supplemental material for

D-Amino Acids Inhibit Initial Bacterial Adhesion: Thermodynamic Evidence

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Part 1: Methods and Materials

Bacterial size. The size of bacteria was calculated using a scanning electron microscope (SEM, HITACHI S-570, Japan). The cells were fixed with 3.0% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), dehydrated with ethanol, silver-coated by a sputter, and observed in the SEM.

Bacterial preparation of Zeta potential. Zeta potential (ζ potential) of the bacteria (ZetaSizer 3000HSA (Malvern, England)) was determined using freshly harvested cells from the LB media with different concentrations of D-tyrosine (0, 10, 25, 50 μM) and resuspended in 10 mM KCl (the pH of the solution was unadjusted (5.6-5.8) at an optical density of 0.2-0.25 measured at 600 nm with a spectrophotometer.

Cells Adhesion and Desorption Tests

Cells adhesion tests were conducted as previously reported (Kim et al. 2009). The attachment efficiency (A, %) was calculated as:

$$A(\%)=\frac{C_0-C_e}{C_0} \quad \text{(S1)}$$

where C_o, C_e are the initial and final optical density, respectively.

Next the desorption rate (R, %) (OD_{600} of reversibly adhered bacteria divided by the value of OD_{600} for all the adhered bacteria) was calculated for each test by experimentally determined C_o' (OD_{600} before desorption tests in 0.1 mM KCl solution) and C_e' (OD_{600} after desorption tests in 0.1 mM KCl solution) as follows (Kuznar and Elimelech 2007):

$$R=\frac{C_e'-C_0'}{C_0-C_e}\times 100\% \quad \text{(S2)}$$

If adhesion of the cells was completely irreversible then R = 0%, whereas if R = 100% adhesion was completely reversible. All the experiments were conducted for at least three times.

Part 2: Equations and parameters of surface thermodynamics

The surface tension component and parameters of bacterial surface were calculated with eq. S3:

$$(1+\cos\theta)\gamma_L = 2((\gamma_B^{LW}\gamma_L^{LW})^{1/2} + (\gamma_B^{+}\gamma_L^{-})^{1/2} + (\gamma_B^{-}\gamma_L^{+})^{1/2}) \quad \text{(S3)}$$

where θ is the contact angle between the bacteria surface and the drop liquid and L represents the liquid used in the experiment. γ^+ and γ^- are the electron-acceptor and electron-donor parameters, respectively. The γ^+, γ^- and γ^{LW} of bacteria could be determined by eq. S3.

The parameters for eq. 4 - 6 (shown in the manuscript) are listed here:

$$\Delta G^{LW} = 2(\sqrt{\gamma_L^{LW}} - \sqrt{\gamma_G^{LW}})(\sqrt{\gamma_B^{LW}} - \sqrt{\gamma_L^{LW}}) \quad \text{(S4)}$$

$$\Delta G^{AB} = 2\sqrt{\gamma_L^{+}}(\sqrt{\gamma_G^{-}} + \sqrt{\gamma_B^{-}} - \sqrt{\gamma_L^{-}}) + 2\sqrt{\gamma_L^{-}}(\sqrt{\gamma_G^{+}} + \sqrt{\gamma_B^{+}} - \sqrt{\gamma_L^{+}}) - 2(\sqrt{\gamma_G^{+}\gamma_B^{-}} + \sqrt{\gamma_G^{-}\gamma_B^{+}}) \quad \text{(S5)}$$

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Part 3: Results

TABLE S1 Total interaction energy profiles as a function of separation distance between *E. coli* JM109 cells and quartz sand

Parameters	**0 μM**	**10 μM**	**25 μM**	**50 μM**
Secondary Energy Minima Depth (kT)	(-)[a]	-114.5	-54.7	-54.1
Secondary Energy Minima Distance (nm)	(-)[a]	1.7	2.9	3.1
Closest Approach Distance (nm)	(-)[a]	0.6	2.0	2.2
Hamaker Constant (J)	3.3×10^{-21}	3.2×10^{-21}	3.1×10^{-21}	2.9×10^{-21}

56 (-)[a] indicate the values that do not exist.

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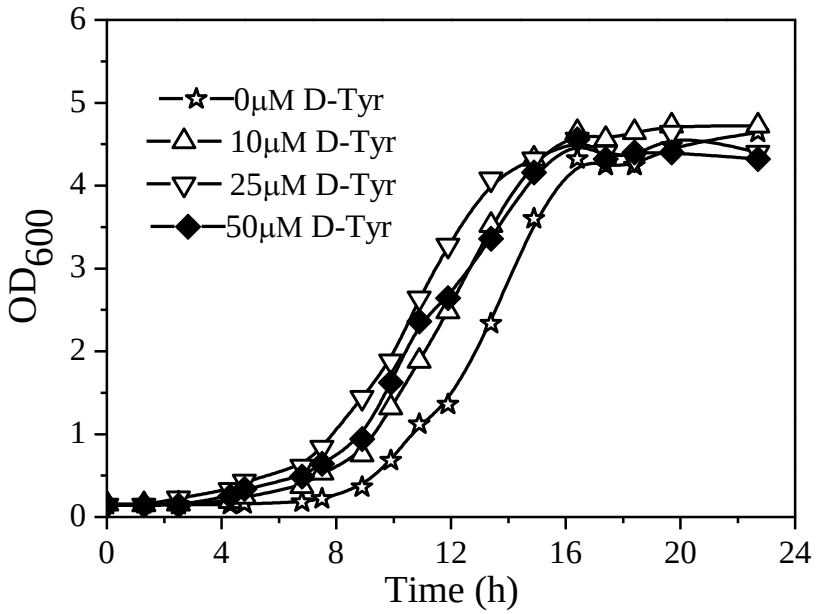
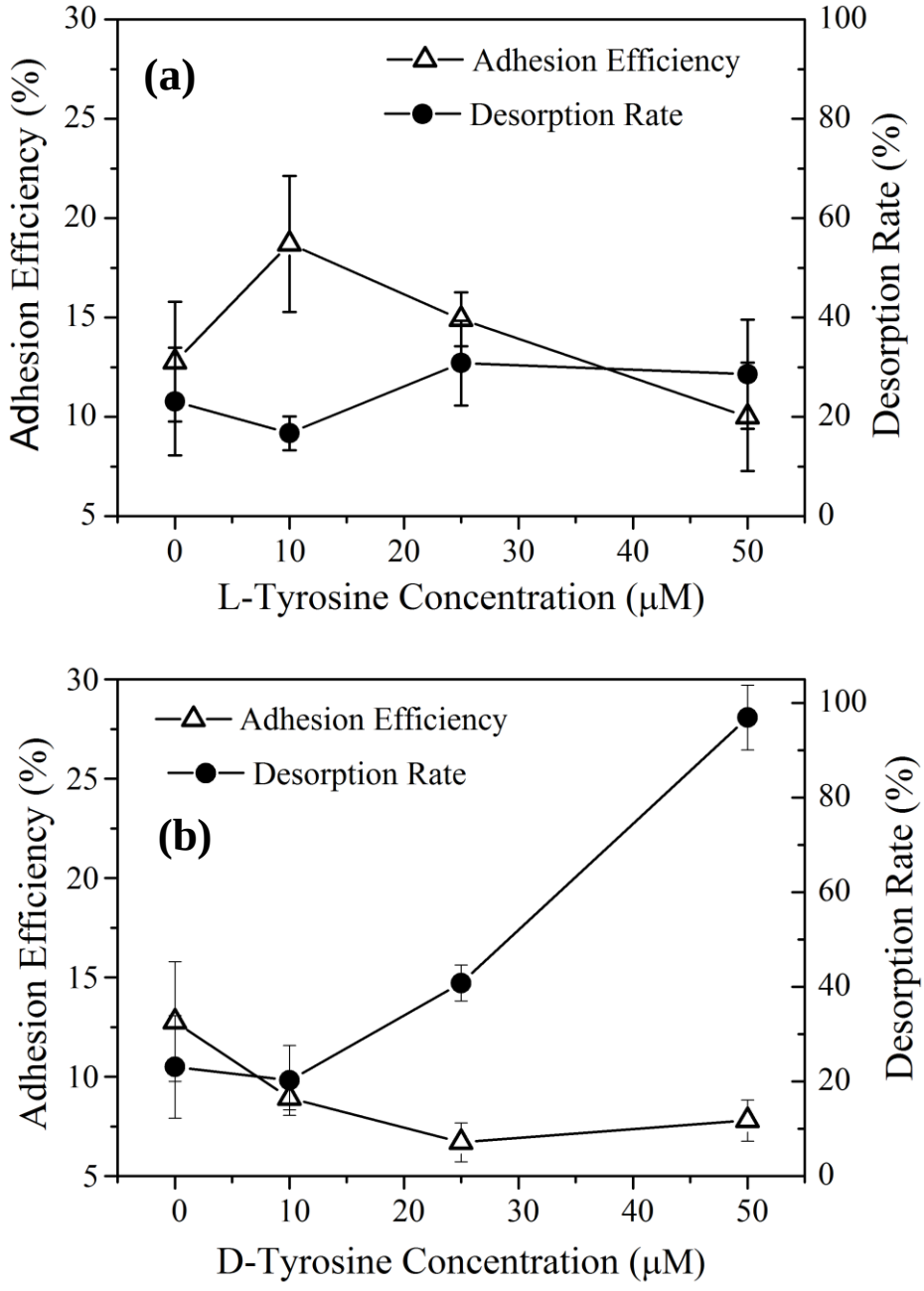


Figure S1. Growth curves of *E. coli* cells from LB media with different D-Tyrosine concentrations (0, 10, 25, 50 μM).



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63 **Figure S2.** Adhesion and desorption efficiencies of E. coli onto and off of a quartz

64 collector surface, determined as a function of D-Tyrosine (a), and L-Tyrosine (b).

65 Experiments were conducted at unadjusted pH (5.6-5.8), and at room temperature

66 (25°C); bacteria were cultivated from the bacterial minimal media. Error bars indicate

67 one standard deviation.

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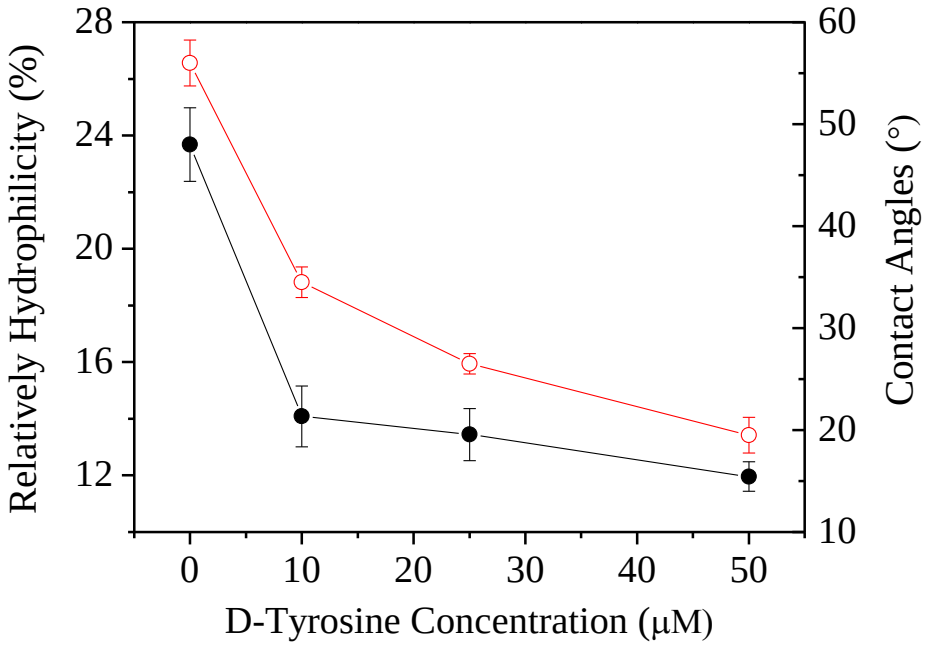

Figure S3. The relative hydrophobicity and contact angles of *E. coli* cells as a function of D-Tyrosine concentration.

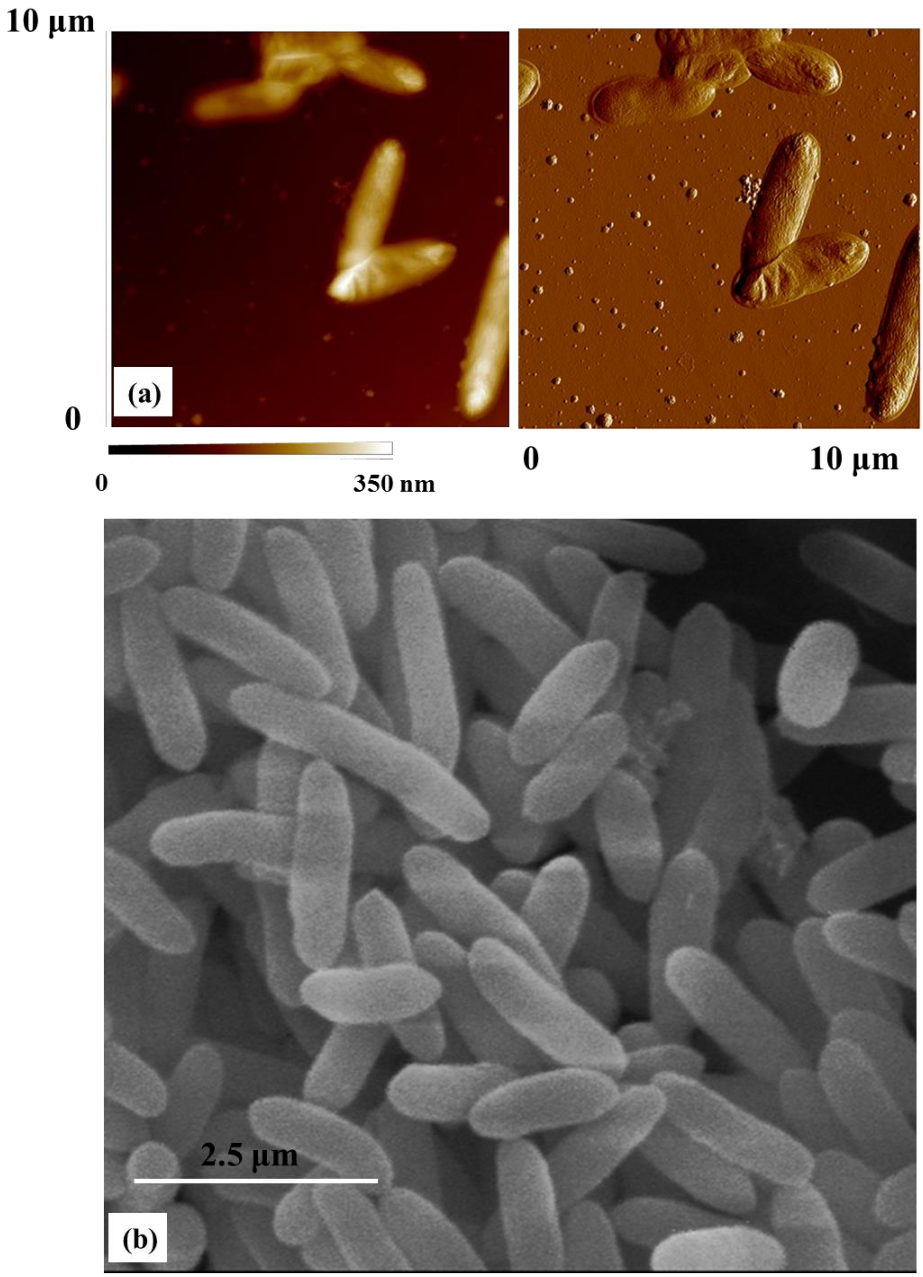

Figure S4. AFM topographical (left) and deflective (right) images are shown for (a) *E. coli* JM109 cells and (b) SEM images of *E. coli* JM109 cells.

References

Kim HN, Hong Y, Lee I, Bradford SA, Walker SL. 2009. Surface characteristics and adhesion behavior of *Escherichia coli* O157:H7: Role of extracellular macromolecules. Biomacromolecules 10: 2556-2564.