# Supplemental Material for geometry-driven collective ordering of bacterial vortices

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**Bacterial culture**

Bacteria *Escherichia coli* RP4979 strain that was deficient of tumbling ability due to the lack of *cheY* gene was used. RP4979 bacterial strain was transformed with a plasmid DNA that encodes constitutive expression of YFP protein. YFP expression allows to test spatial homogeneity of bacteria in microwells. We inoculate single colony of RP4979 in LB medium (NaCl 10 g/L, Tryptone 10 g/L, Yeast extract 5 g/L, autoclaved at 120 °C for 20 min) with selective antibiotics (25 µg/mL chrolamphenicol) and then have bacteria grow at 37 °C with shaking 150 rpm. The overnight culture was diluted by a factor of 100 in T-broth (NaCl 10 g/L, Tryptone 10 g/L, autoclaved at 120 °C for 20 min) of 50 mL with selective antibiotics and diluted culture was incubated at 30 °C with shaking at 150 rpm until bacterial density reached $O.D._{600}$=0.4. Grown culture was centrifuged at 3000 rpm for 10 min and the supernatant was removed carefully in order to increase the volume fraction of bacteria to 20%(v/v).

In addition, we used elongated bacteria that were prepared by the exposure to 20 µg/mL of cephalexin (CEP), an inhibitor of bacterial cell division, for 1 hour just before the end of cultivation. The elongated bacteria tends to form larger vortex as shown in FIG. 1. Bacteria used in this study were transformed with a plasmid encoding YFP protein and its YFP fluorescence allows to measure the size of individual bacteria accurately. We analyzed the size of individual bacteria by conventional image processing and the averaged size of long axis is linearly increased with the duration of CEP treatment. The velocity of bacteria after CEP treatments for various exposure durations was measured as the displacement of the center of mass. The speed of bacterial motion is comparable to that of untreated bacteria. However, we found that the maximum speed in the velocity field after PIV is 9.4±2.0 µm/s for CEP treated bacteria, which is comparable or slightly faster than the maximum speed of 8.4±0.2 µm/s in PIV for untreated bacteria. This difference in velocity may result from the hypothetical correlation among the size of bacteria, the size of vortices, and the alignment of bacteria but it is out of the focus of this study.

**Microfabrication**

We used SU-8 3025 photoresist (Microchem) for all the photolithographies necessary in this study. Chromium masks (MITANI micronics, Japan) were used to print patterns in the photoresist during an exposure to UV light in a mask-aligner (MA-100, MIKASA, Japan). Molds of poly-dimetyhl siloxane (PDMS) microwells were cured on the surface of silicon wafers, while the surface of SU-8 patterns was smoothed by coating with CYTOP, a fluorinated coating agent (Asahi glass, Japan). PDMS elastomer was cast on top of the patterned SU-8 mold and cured at 70 °C for 1 hour. The hardened PDMS was cut with a scalpel and the patterned surface was coated with MPC polymer (Lipidure, Nichiyu Coop., Japan) and heated for 1 hour at 50 °C, which increased its hydrophilicity, to avoid non-specific adhesion of bacteria. The thickness of the PDMS microwells was measured by laser scanning surface profiler (LT-9000, Keyence, Japan) and it was about 20 µm. The glass cover slips used as the bottom of the microfluidic chips were also coated with MPC according to the same recipe in order to avoid the non-specific adhesion of bacterial bodies.

**Image acquisition and processing**

0.5 µL of dense bacterial suspension was put onto the MPC-coated coverslip. Thereafter, MPC-coated PDMS microwells were placed on top of the droplet and then pressed to enclose bacterial suspension. Bright-field optical imaging and video-microscopy were performed using an inverted microscope (IX73, Olympus). We recorded swarming motion of bacteria at a rate of 30 fps with a CCD camera (DMK23G445, Imaging Source) controlled by custom made LabVIEW program. The experimental data was acquired within 30 min after preparing dense bacterial suspension in order to avoid the proliferation (doubling time is about 1.5 h in T-broth) and to use fresh bacteria without losing motility. Velocity fields of bacterial swarm were obtained by PIV with Wiener filter method using PIVlab based on MATLAB software. Acquired velocity fields were further smoothed by averaging over 30 frames that correspond to 1 s. To analyze disordered state of bacterial vortices as shown in FIG. 1(a), we calculated energy spectrum $E(k)$ of two-dimensional space that indicates the kinetic energy at the wavenumber $k = 2\pi/r$. Two-dimensional energy spectrum can be obtained by Fourier transform of two-point velocity correlation function as $E(k) = \frac{k}{2\pi} \int d^2r' e^{-ik\cdot r'} \langle v(r,t) \cdot v(r+r',t) \rangle$ where $r'$ is the distance between two arbitrary points for the calculation of velocity correlation function at the same time point $t$[1].

**Model of the transition of vortex pairing**

Here we consider self-propelling point-like particles that can interact through a potential $U$ of polar alignment. The state of particle $m$ at time $t$ is represented by two variables, its coordinate $\boldsymbol{x}_m(t)$ and its orientational angle of motion $\theta_m(t)$. Particles align their direction of motion through $\partial_\theta U(\boldsymbol{x}_m, \theta_m)$ and their relaxation coefficient is given by $\gamma$. Hence, the evolution of $\boldsymbol{x}_m(t)$ and $\theta_m(t)$ belong to a Vicsek-like model as follow:

$$\dot{\theta}_m = -\gamma \frac{\partial U}{\partial \theta_m} + \eta_m(t) \tag{1}$$

where $\eta_m(t)$ is random noise, which means that the direction of motion of the particles is random at infinite dilution limit, and its correlation satisfies $\langle \eta_m(t) \rangle = 0$, $\langle \eta_m(t)\eta_n(t') \rangle = 2D\delta_{mn}\delta(t-t')$ where $\delta_{mn}$ and $\delta t$ is Dirac delta function. $D$ is the diffusion constant in rotational direction, which is related to noise strength.

In addition, for two-dimensional coordinate,

$$\dot{\boldsymbol{x}}_m = v_0 \boldsymbol{e}(\theta_m) \tag{2}$$

where $\boldsymbol{e}(\theta_m)$ is unit vector of velocity defined as $\boldsymbol{e}(\theta_m) = (\cos\theta_m, \sin\theta_m)$. We can easily find that particles move at a constant speed $v_0$ while fluctuation is involved in rotational direction alone.

The alignment of velocity vector is based on polar interaction and hence the potential $U(\boldsymbol{x}_m, \theta_m)$ is

$$U(\boldsymbol{x}_m, \theta_m) = -\sum_{|\boldsymbol{r}_{mn}|<\epsilon} \cos(\theta_m - \theta_n) \tag{3}$$

where $\boldsymbol{r}_{mn} = \boldsymbol{x}_m - \boldsymbol{x}_n$ and $\epsilon$ is the effective radius of particle interaction.

We consider a distribution of particles showing homogeneous spatial distribution. Namely, probability distribution is a function of $\theta$ and $t$. For this case, Fokker-Planck equation of the point-like particles is given by

$$\frac{\partial P}{\partial t} = D\frac{\partial^2 P}{\partial \theta^2} + \gamma \frac{\partial}{\partial \theta}\Big( \int_{-\pi}^{\pi} \sin(\theta - \theta') P(\theta', t) d\theta' P(\theta, t) \Big) \tag{4}$$

where $P(\theta, t; \phi)$ is the probability distribution of particles heading $\theta$ at time $t$. The focus of this theoretical analysis is to find analytical solution that can account for the transition from FMV to AFMV observed in experiment. In that sense, what we need to consider is the interaction of particles from left or right circles in a doublet microwell defined by geometric constant $\phi$. As for this case, the Fokker-Planck equation can be expressed by

$$\frac{\partial P}{\partial t} = D\frac{\partial^2 P}{\partial \theta^2} + \gamma \frac{\partial}{\partial \theta}\Big( \int_{-\pi}^{\pi} \sin(\theta - \theta') \bar{P}(\theta', t; \phi) d\theta' P(\theta, t; \phi) \Big) \tag{5}$$

where $\bar{P}(\theta', t; \phi)$ is the probability distribution of the orientation of particles $\theta'$ at the tip from either left or right circle. Hence, the probability distribution $P(\theta, t; \phi)$, meaning the orientation angle $\theta$ of particles rectified by the polar alignment at the tip, is able to be derived as the analytical solution of Eq. (4) once we get the explicit form of $\bar{P}(\theta', t; \phi)$. Therefore, we next consider the motion of particles due to the association with boundary wall to find the form of $\bar{P}(\theta', t; \phi)$.

The interaction between motile particles $m$ and the wall $\bar{n}$ is assumed nematic. Fokker-Planck equation of the heading $\theta$ of particles associated with the boundary is given by

$$\frac{\partial \bar{P}}{\partial t} = \bar{D}\frac{\partial^2 \bar{P}}{\partial \theta^2} + \bar{\gamma} \frac{\partial}{\partial \theta}\Big( \int_{-\pi}^{\pi} \sin\big(2(\theta - \theta')\big) \bar{P}(\theta', t) d\theta' \bar{P}(\theta, t) \Big) \tag{6}$$

$$\bar{U}(\boldsymbol{x}_m, \theta_m) = -\sum_{|\boldsymbol{r}_{m\bar{n}}|<\bar{\epsilon}} \cos\big(2(\theta_m - \theta_{\bar{n}})\big) \tag{7}$$

where $\boldsymbol{r}_{m\bar{n}} = \boldsymbol{x}_m - \boldsymbol{x}_{\bar{n}}$ and $\bar{\epsilon}$ represents the range of the effective nematic interaction between a particle $m$ and a wall $\bar{n}$. We note that a vortex in a circular microwell and vortex pairing patterns (FMV and AFMV) in a doublet

microwell are persistent in time within the range of measurement. This fact allows us to consider the steady state, $\partial_t P = 0$ and $\partial_t \bar{P} = 0$, to analyze Eqs. (5) and (6), respectively. The solution of Eq.(6) at the steady state is

$$\bar{P}(\theta) = \frac{1}{2\pi I_0(\alpha\bar{\gamma}/\bar{D})} \exp\left[\frac{\alpha\bar{\gamma}}{\bar{D}} \cos 2(\theta - \theta_0)\right] \tag{8}$$

where $\alpha = \int_{-\pi}^{\pi} \cos(2\theta)\bar{P}(\theta)d\theta$ and $I_0(x)$ is modified Bessel function of the first kind and $\theta_0$ is the tangential angle at the boundary. Close to the boundary, the nematic interaction with the wall is assumed strong enough to neglect the angular noise, so that $\bar{\gamma}/\bar{D} \to \infty$. The condition of low noise reflects the state which $\bar{P}(\theta)$ is no longer constant and thereby one can find $\alpha \neq 0$. The probability distribution is rewritten as

$$\lim_{\bar{\gamma}/\bar{D}\to\infty} \frac{\exp\left(\frac{\alpha\bar{\gamma}}{\bar{D}} \cos 2(\theta - \theta_0)\right)}{2\pi I_0(\alpha\bar{\gamma}/\bar{D})} = \delta(\theta - \theta_0 - l\pi) \tag{9}$$

where $\delta(\theta)$ is the Dirac delta function and $l$ is $0, \pm 1, \pm 2, \cdots$ but $\delta(\theta - \theta_0)$ and $\delta(\theta - \theta_0 - \pi)$ are taken to describe either clockwise or counter-clockwise motion along the boundary for later analysis. Thus, the explicit form of $\bar{P}(\theta'; \phi)$ can be obtained by considering the tangential direction of the curved boundary at the tip.

As for a doublet of circular microwells (Dcm) with geometrical parameter $\phi$, given that particles enter into left microwell by either incoming or outgoing direction at the tip, the probability of particle heading $\theta'$ from left is given by

($I$) Outgoing from left microwell

$$\bar{P}(\theta'; \phi) = \delta(\theta' - \pi/2 + \phi), \tag{10}$$

or

($I^*$) Incoming into left microwell

$$\bar{P}(\theta'; \phi) = \delta(\theta' + \pi/2 + \phi). \tag{11}$$

where we use the relation $\theta_0 + \phi = \pm\pi/2$ nearby the tip of Dcm. The particles that move along the boundary of a doublet microwell interact close to the tip. Hence, in addition to Eqs. (10) and (11), one needs to take the bacterial motion from the right side into account so as to describe the collective motion after the association between particles coming from both left and right sides. Hence, the probability of particle heading $\theta'$ from right is given by

($II$) Outgoing from right microwell

$$\bar{P}(\theta'; \phi) = \delta(\theta' - \pi/2 - \phi), \tag{12}$$

or

($II^*$) Incoming into right microwell

$$\bar{P}(\theta'; \phi) = \delta(\theta' + \pi/2 - \phi). \tag{13}$$

On the one hand, as shown in FIG. 4(a), one can assume that particles can form AFMV pattern when polar interaction of (($I$) and ($II$)) or (($I^*$) and ($II^*$)) dominantly occurs at the middle. On the other hand, FMV pattern results from polar interaction of (($I$) and ($II^*$)) or (($I^*$) and ($II$)) because the group of particles keep moving along boundary wall. Therefore, by taking one pair of two explicit forms $P(\theta'; \phi)$ given above, one can solve Fokker-Planck equation of Eq. (5) and finally obtain the probability distribution of particle heading $\theta$ at the tip, as given in Eqs. (3) and (4) in main text.

**Velocity of a vortex in a circular microwell**

In this section, we derive the function of angular velocity of single vortex, $v_\theta(r)$, formed inside a circle of the radius $R$. We assume that velocity of bacterial swarming decays at the vicinity of boundary wall so that the boundary condition at $r = R$ is $v_\theta(r)$=0. However, $v_\theta(r)$ is proportional to $r$ and does not satisfy the above condition if one supposes uniform vorticity inside the circle $r < R$. To reconcile both vortex formation and the boundary condition at $r = R$, the superposition of two different vortices has to be taken into account. Indeed, FIG. 1(d) exhibits the presence of two regions with opposite vortices. Hence, the spatial distribution of vorticity inside the circle is given by

$$\omega(r) = \begin{cases} \omega & (0 \leq r \leq R - s) \\ -\omega\left[1 - \frac{(R-s)^2}{R^2}\right] & (R - s \leq r \leq R) \end{cases} \tag{14}$$

where $R-s$ is the position we find the peak of angular velocity. By solving Laplace equation, the analytic expression of the orthoradial velocity in a circular microwell $\boldsymbol{v}(r,\theta)$=$v_\theta(r)\boldsymbol{t}(\theta)$ can be obtained as

$$\boldsymbol{v}(r,\theta) = \begin{cases} \frac{\omega}{2}\left[1-\frac{(R-s)^2}{R^2}\right] r\boldsymbol{t}(\theta) & (0 \le r \le R-s) \\ \frac{\omega}{2}\left(1-\frac{s}{R}\right)^2 \frac{R^2-r^2}{r}\boldsymbol{t}(\theta) & (R-s \le r \le R) \\ 0 & (r > R) \end{cases} \tag{15}$$

where $\boldsymbol{t}(\theta) = (-\sin\theta, \cos\theta)$ is the unit orthoradial vector at the angular position $\theta$. The quantity $s$ is 4.6 µm estimated from experimental data. In the following section, this analytic formulation is used to define the order parameter of AFMV pattern.

**Order parameter of AFMV pattern**

Here we show the derivation of order parameter of anti-ferromagnetic vortices (AFMV) pattern, given by Eq. (1) in main text. This order parameter compares the matching between the observed pattern of vortex pair in experiments and numerically calculated AFMV. For the numerical calculation of AFMV, the phenomenological description of vortex confined in boundary is considered as follows: for each circle composing the doublet microwell, we set an index $j$, 1 stands for the left side and 2 for the right side. We define two sets of polar coordinates $(r_j, \theta_j)$; one for left circle is $(r_1, \theta_1)$ and the other for right circle is $(r_2, \theta_2)$. The origin of $j$ polar coordinates is set at the center of $j$ circle. We consider $\boldsymbol{t}_j(\theta_j)$ the base polar orthoradial vector at the angular position $\theta_j$ centered on the center of the circle $j$ for $0 \le r_j \le R$. In particular, we have $\boldsymbol{v}_j(r_j,\theta_j) = v_\theta(r_j)\boldsymbol{t}_j(\theta_j)$ where $v_\theta(r_j)$ is given by Eq. (15) and $\omega$ is the vorticity discussed at the previous section.

We then consider vortices showing AFMV pattern in the doublet microwell. In addition to the boundary condition of a doublet of circles that is characterized by $R$ and $\Delta$, the polar coordinates $(r,\theta)$ is given to define the internal space. The origin of polar coordinates is placed at the centroid of the doublet shape. The velocity field, $\boldsymbol{v}(r,\theta)$, is in turn considered as the superposition of two vortices in $j$=1 and 2 circles. Because two vortices in AFMV pattern show opposite angular velocities, we can write $\boldsymbol{t}_1(\theta) = -\boldsymbol{t}_2(\theta)$ and then describe the velocity field as

$$\boldsymbol{v}(r,\theta) = \sum_j \boldsymbol{v}_j(r_j,\theta_j) = \sum_j v_\theta(r_j)\boldsymbol{t}_j(\theta_j). \tag{16}$$

The expected streamline of an AFMV pattern with a velocity field $\boldsymbol{v}(r,\theta)$ lies on the unit vector $\boldsymbol{u}(r,\theta)$ such that

$$\boldsymbol{u}(r,\theta) = \frac{\boldsymbol{v}(r,\theta)}{|\boldsymbol{v}(r,\theta)|}. \tag{17}$$

To describe the transition between FMV and AFMV patterns, we consider the deviation from the expected AFMV pattern given by the product of expected velocity orientation map $\boldsymbol{u}(r,\theta)$ and the one measured experimentally $\boldsymbol{p}(r,\theta)$. The order parameter $\Phi$ is then defined as

$$\Phi = |\langle \boldsymbol{p}(r,\theta)\cdot\boldsymbol{u}(r,\theta)\rangle| \tag{18}$$

where $\langle\cdot\rangle$ denotes the ensemble average over all sites in a doublet microwell. One can find $\boldsymbol{p}(r,\theta)\cdot\boldsymbol{u}(r,\theta) = \cos(\psi(r,\theta) - \psi^0(r,\theta))$ where $\psi(r,\theta)$ and $\psi^0(r,\theta)$ are the orientational angles of $\boldsymbol{p}(r,\theta)$ and $\boldsymbol{u}(r,\theta)$, respectively. When an actual AFMV pattern is recorded in $\boldsymbol{p}(r,\theta)$, $\Phi$ is close to 1, while for an FMV pattern, it is close to 0.

**Supplemental movies**

We provide three movies in video supplemental material:
(Video1) A single vortex formation in a circular microwell,
(Video2) Ferromagnetic-like vortex (FMV) pairing in a doublet of circular microwells,
(Video3) Anti-ferromagnetic-like vortex (AFMV) pairing in a doublet of circular microwells.

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