Supporting Information

A Platform for Stop-Flow Gradient Generation to Investigate Chemotaxis

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Supporting information

Experimental Section

Janus microswimmers preparation:
A monolayer of SiO₂ microspheres was obtained by a Langmuir-Blodgett method. Cu@SiO₂ Janus micromotors were prepared by depositing a 30 nm thick layer of Cu on the top of a SiO₂ monolayer by thermo-deposition. All Janus particles were collected by sonication and resuspended in deionized water.

Microfluidic setup and chemotaxis assay:
The microfluidic setup for gradient generation and stop flow is illustrated in figure 1A. It is composed of one OB1 Mk3+ pressure controller, one MUX Wire valves controller, two flow sensors MFS4D, three microfluidic valves type 3/2 (Elveflow, France) and one microfluidic chip (Fluidic 172, Microfluidic Chip Shop GmbH, Germany). The tubing connecting the different components to the chip is in PTFE and has an internal diameter of 794 µm. The pump and valves are controlled by the ESI software from Elveflow and a sequence was created to automatically actuate the valves and correct the pressure for an instantaneous stop flow. During flow conditions, the flow rates were kept constant thanks to a feedback loop from the flow sensors to the pressure pump, as the pressure was continually adjusting to compensate any deviations in flow rate.

Figure 1: Description of the automated sequence used to perform the stop flow.
**Video recording:**  
The movement of micromotors was observed under a Carl Zeiss microscope. Videos are taken and recorded by a camera connected to the microscope at a frame rate of 30 fps.

**Data analysis:**  
The videos are processed and analyzed by MATLAB to coordinate moving particles and calculate the instantaneous speeds and moving angles of them.

**Gradient generation model with fluorescein**

![Image of gradient generation model with fluorescein](image)

Figure 2: Experimental data of the gradient of fluorescein evolution over 10 min after stop flow. (A) Fluorescence imaging at $t = 0$ min and $t = 10$ min. (B) Normalized fluorescent intensity profile at different time intervals.
Orientation angles analysis

Figure 3: Normalized histogram of orientation angles in chemical gradient at different concentrations of H$_2$O$_2$ (A)-(C) and no gradient at 2.5% (D) as a contrast.

Figure 4: Average speeds of chemotaxis at different concentrations of H$_2$O$_2$.

Numerical simulation

All the simulations were implemented by COMSOL Multiphysics package (version 5.6) with a 2D configuration.

Diffusion model:
To investigate the concentration distribution of H$_2$O$_2$ in the microfluidic chip, we built a dilute species transport model to simulate the diffusion of H$_2$O$_2$ at different flow rates and time intervals. A chip-shaped geometry is set to be a calculated domain and fulfilled with water.
In the transport of diluted species module, the diffusion of H$_2$O$_2$ is governed by the general flux equation (1):

$$\nabla J = u \cdot \nabla c - D \nabla^2 c - \frac{zFD \cdot (c \nabla V)}{RT}$$  \hspace{1cm} (1)

where $J$ is the flux of species H$_2$O$_2$ in this simulation, and the three terms on the right represent convection, diffusion, and migration, respectively. Here $u$ is the fluid velocity, $V$ is the electrostatic potential, $R$ is the gas constant, $F$ is the Faraday constant, $T$ is the temperature and $c$, $D$, $z$ are the concentration, diffusion coefficient and charge of species, respectively. Since H$_2$O$_2$ is electrically neutral in water, the migration contributes to the final distribution is negligible.

We used a nonlinear stationary solver to solve the distribution at different flow rates and a time-dependent solver for the different time intervals.

**Self-electrophoresis model:**

To simulate the electrophoresis of Cu@SiO$_2$ particles in H$_2$O$_2$, in this model, a sphere of 5 µm in diameter is placed in the center of a cube box with a length of 100 µm. The cube is set to be a calculated domain and fulfilled with water. The distribution of electric potential and fluid flow field of the domain is solved with a nonlinear steady solver implemented in COMSOL.

Based on the previous work, we assumed a self-electrophoresis mechanism of the particle.[1] The oxidation and reduction of H$_2$O$_2$ occur preferentially at the equator and pole of the copper cap respectively.

In electrostatic module, the calculated domain is governed by the following equation (2):

$$E = -\nabla V$$  \hspace{1cm} (2)

where $E$ is electric field in space and $V$ is electric potential. $E$ is further solved by the space charge density at each point in the domain by (3):

$$\nabla \cdot (\varepsilon E) = \rho_V$$  \hspace{1cm} (3)

Where $\varepsilon$ is medium electrical permittivity and $\rho_V$ is space charge density.

According to the literature[2], the anode face with an outward flux carries a extrinsic surface charge density $\rho_a$ by (4):

$$\rho_a = \varepsilon E_a = \frac{J_a K_B T}{2e n_0 D_{H^+}}$$  \hspace{1cm} (4)

Where $\varepsilon$ is the medium electrical permittivity, $n_0$ is the bulk proton concentration, $J_a$ is the ionic flux on anode.

For a particle moving in a uniform H$_2$O$_2$ bulk solution, $J_a$ is equal everywhere on the anode surface. As the particle placed in H$_2$O$_2$ with a linear gradient distribution, $J_a$ is derived as the following equation (5):

$$J_a = \nabla J_{a0} \cdot R \cos \theta$$  \hspace{1cm} (5)

where $J_{a0}$ is the maximum flux on the anode surface, $R$ is the radius of the particle, $\theta$ is the angle between the Janus interface of the particle and the H$_2$O$_2$ gradient direction.

The cathode with an inward flux carries an opposite surface charge density $\rho_c$ by (6),

$$\rho_c = \varepsilon E_c = \frac{J_c K_B T}{2e n_0 D_{H^+}}$$  \hspace{1cm} (6)

Where $J_c$ is the flux on cathode.

As the same to the anode, $J_c$ is uniform on the cathode surface with no gradient. In a linear gradient situation, $J_c$ follows the below equation (7):

$$J_c = \nabla J_{c0} \cdot R \cos \theta$$  \hspace{1cm} (7)
where $J_{c0}$ is the maximum flux on the cathode surface.

In the creeping flow module, the calculated domain is governed by the following equation:

$$\nabla \cdot u = 0$$

$$\nabla p = \eta \nabla^2 u$$

Where $p$ is the pressure, $\eta$ is dynamic viscosity of water, and $u$ is the fluid flow velocity. The two modules are coupled by an electroosmotic boundary condition on the particle and substrate surface:

$$U_{eo} = \frac{\zeta \epsilon}{\eta} E_{\tan}$$

Where $U_{eo}$ is the electroosmotic speed of fluid on the particle and substrate surface, $\zeta$ is the zeta potential (SiO$_2$ surface set to be -44 mV, Cu surface set to be +38 mV according to measurements), and $E_{\tan}$ is the tangential component of the electric field that is solved by the (2) and (3).

**Supporting videos**

Video S1: Cu@SiO$_2$ micromotors stopped immediately when a zero flow is applied.
Video S2: Cu@SiO$_2$ micromotors moving in a H$_2$O$_2$ gradient after a zero flow is applied.
Video S3: Cu@SiO$_2$ micromotors moving in H$_2$O$_2$ with no gradient.
Video S4: Cu@SiO$_2$ micromotors drifted when a zero flow is not applied precisely.

**References**

[1] X. Lyu, X. Liu, C. Zhou, S. Duan, P. Xu, J. Dai, X. Chen, Y. Peng, D. Cui, J. Tang et al., *Journal of the American Chemical Society* 2021, 143, 12154–12164.

[2] T. R. Kline, J. Iwata, P. E. Lammert, T. E. Mallouk, A. Sen, D. Velegol, *The Journal of Physical Chemistry B* 2006, 110, 24513–24521.