Supporting information for:

Electric field-induced circulation and vacuolization regulate enzyme reactions in coacervate-based protocells

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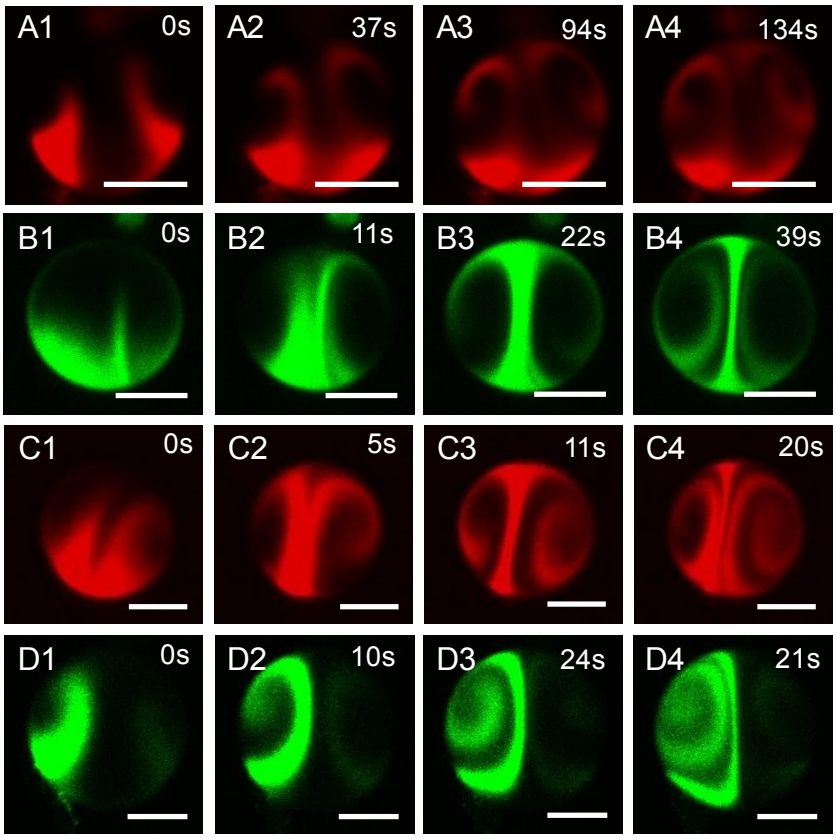

Figure S1. Confocal fluorescence microscopy images showing PLL/ss-oligo coacervate micro-droplets at different charge ratios after addition of Cy5-labelled ss-oligo or FITC-labelled PLL. Circulation is observed in all the cases at E = 10 V cm^{-1}. A1-A4, Cy5 channel, +/-=0.25; B1-B4, FITC channel, +/-=0.25; C1-C4, Cy5 channel, +/-=4; D1-D4, FITC channel, +/-=4. The direction of the electric field (positive to negative) is from

the bottom to top of the images. Scale bar = 10 µm.

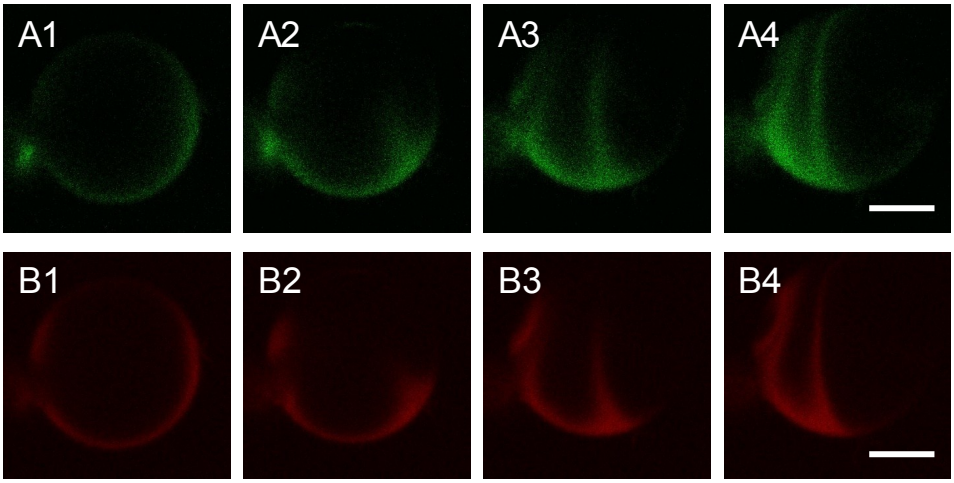

Figure S2: Confocal fluorescence microscopy images showing a time sequence of circulation in a single droplet visualized by FITC-labelled PLL (A1-A4) and Cy5-labelled ss-oligo (B1-B4). The movements of PLL and ss-oligo are synchronized. Cy5-labelled ss-oligo and FITC-labelled PLL were added in sequence. FITC and Cy5 channels were recorded at the same time. E = 10 V/cm. The direction of the electric field (positive to negative) is from the bottom to top of the images. Scale bar, 10 µm.

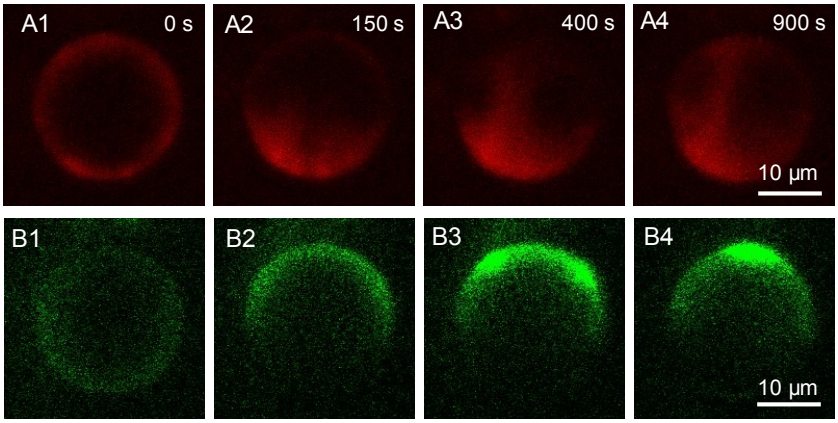

Figure S3: Confocal fluorescence microscopy images showing a time sequence of circulation within a single droplet visualized by Cy5-labelled ss-oligo (A1-A4) and FITC-labelled dextran (B1-B4). Dextran migrates linearly into the droplet (from top to bottom as shown), while ss-oligo undergoes circulation at the same time. Cy5-labelled ss-oligo and FITC-labelled PLL were added in sequence. FITC and Cy5 channels were recorded at the same time. E = 10 V/cm. The direction of the electric field (positive to negative) is from the bottom to top of the images. Scale bar, 10 µm.

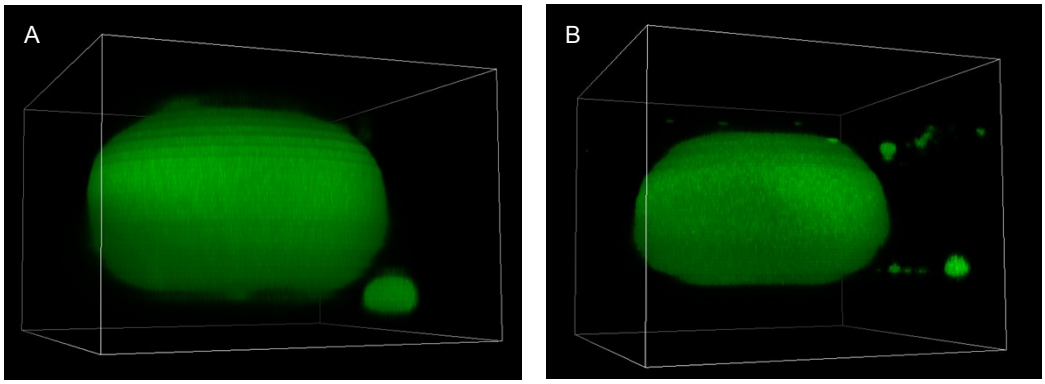

Figure S4: 3D image showing the morphology of the droplet visualized by FITC-labelled PLL before (A) and after the application of electric field at 10 V cm^{-1}.

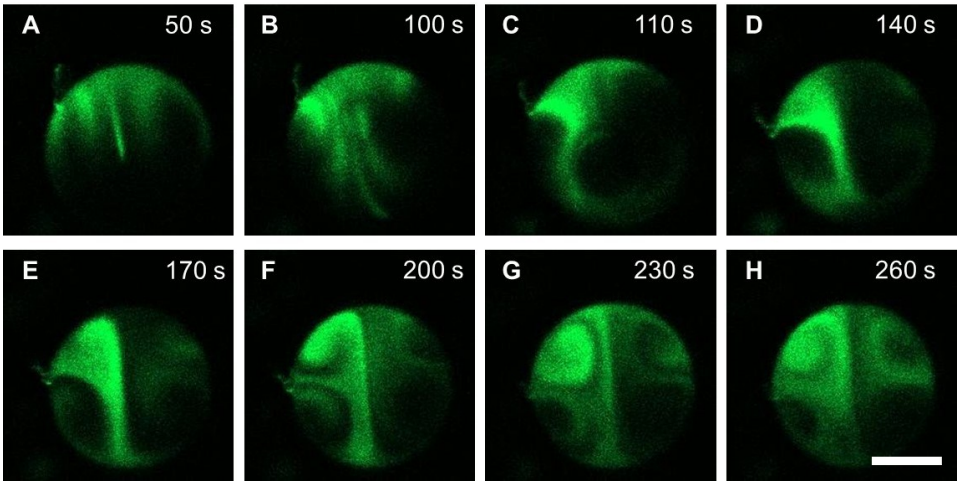

Figure S5: Confocal fluorescence microscopy images showing a bilateral circulation transforming into a quadrupole circulation pattern under E = 10 V/cm. The droplet is visualized by FITC-labelled PLL. The direction of the electric field (positive to negative) is from the bottom to top of the images. Scale bar: 10 μm.

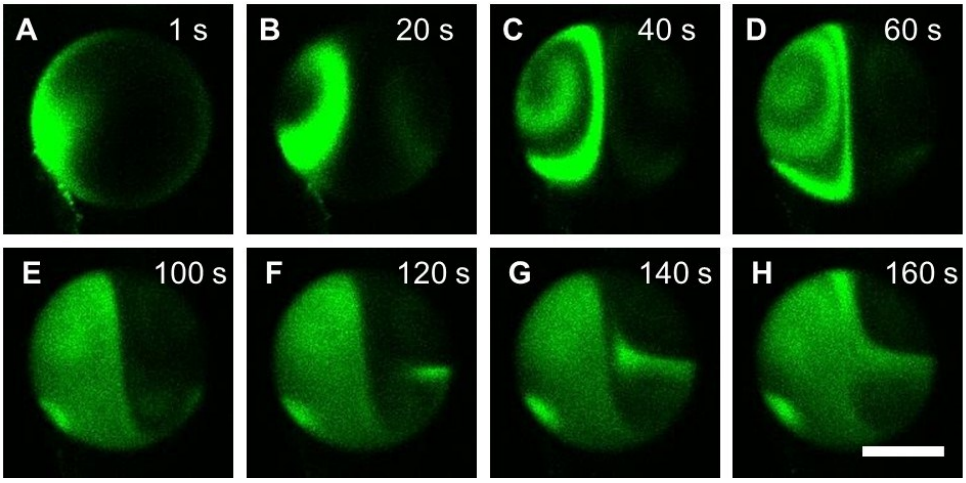

Figure S6: Confocal fluorescence microscopy images showing a bilateral circulation transforming into a triple-pole circulation under E = 10 V/cm. The droplet is visualized by FITC-labelled PLL. The direction of the electric field (positive to negative) is from the bottom to top of the images. Scale bar: 10 μm.

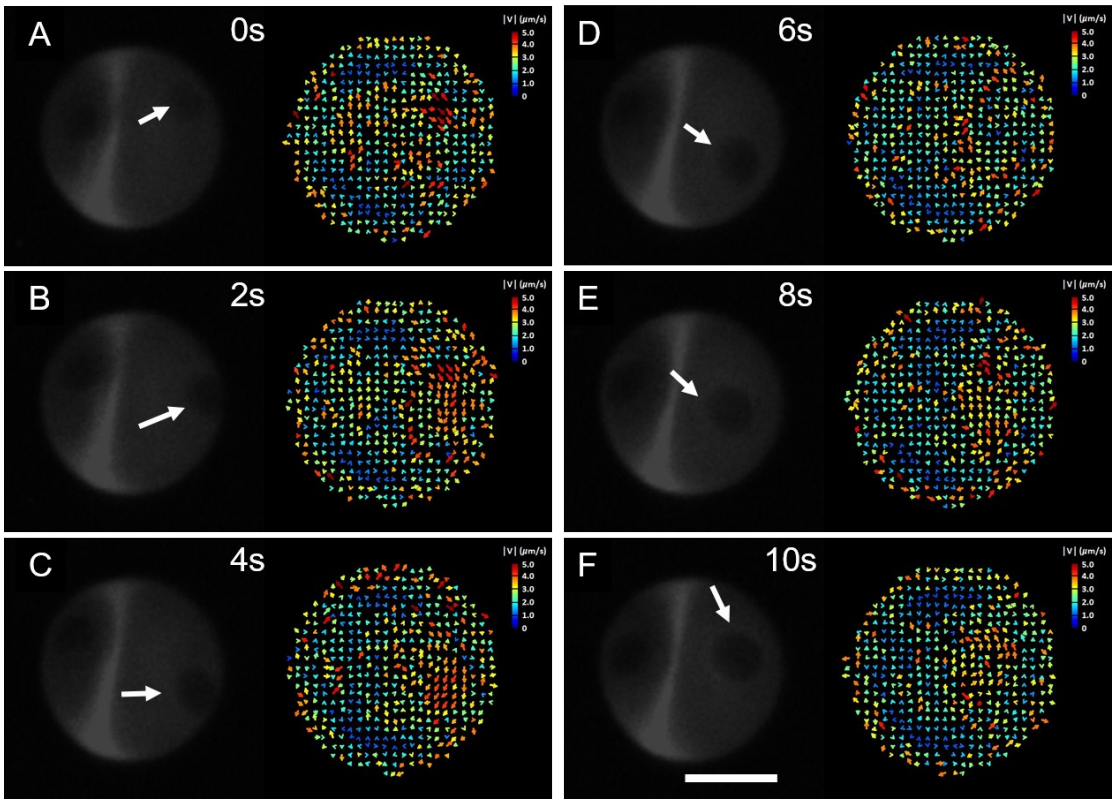

Figure S7. (Left panels) Confocal fluorescence microscopy images showing PLL (2.0 mg mL^{-1})/ss-oligo (0.75 mg mL^{-1}) coacervate droplets stained with Cy5-labelled ss-oligo and energized at E = 10 V cm^{-1}. A vacuole (indicated by arrow) is generated inside one of the circulations. (Right panels) corresponding maps of the flow field showing the velocity for each point in the snapshots. Each arrow indicates the direction of movement at specific positions, and the color of the arrow denotes the velocity with red being the fastest and blue being the slowest. Dramatic changes in movement are highlighted by arrays of elongated red arrows. Data processed by home-built particle image velocimetry. The direction of the electric field (positive to negative) is from the bottom to top of the images. Scale bar, 10 μm.

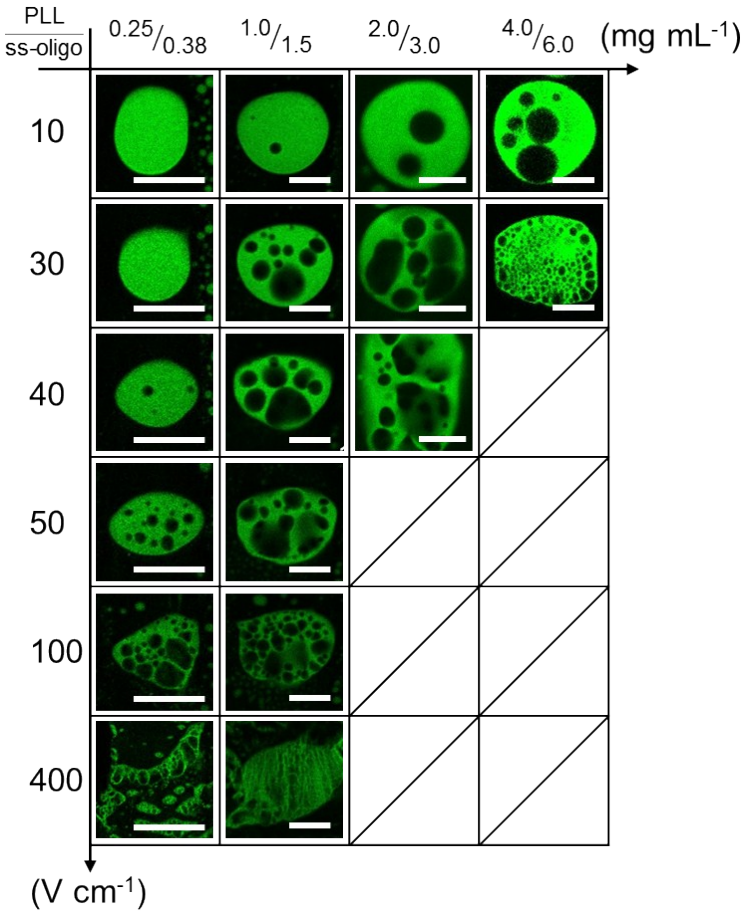

Figure S8: Electric field-induced vacuolization of PLL/ss-oligo coacervate droplets at various PLL and ss-oligo concentrations, and electric fields ranging from 10 V cm^{-1} to 400 V cm^{-1}. The number of vacuoles and their frequency increase with polymer concentration and electric field strength. The size of the vacuoles shows an opposite trend.

Movie S1. Confocal fluorescence microscopy video of the circulation of FITC-labelled PLL in a coacervate droplet confined within a microfluidic channel and exposed to an electric field of 10 V/cm. Movie is shown at 10 times of real time speed at 10 frames per second. Total duration of recording was 14 seconds in real time.

Movie S2. Confocal fluorescence microscopy video of the movement of FITC-labelled PEG into a coacervate droplet confined within a microfluidic channel and exposed to an electric field of 10 V/cm. Movie is shown at 10 times of real time speed at 10 frames per second. Total duration of recording was 10 seconds in real time.

Movie S3. Confocal fluorescence microscopy video of the movement of FITC-labelled PEG into a coacervate droplet confined within a microfluidic channel and exposed to an electric field of 10 V/cm. Cy5-labelled ss-oligo is also added but cannot be detected. Movie is shown at 20 times of real time speed at 20 frames per second. Total duration of recording was 12 seconds in real time.

Movie S4. Confocal fluorescence microscopy video of the circulation of Cy5-labelled ss-oligo in a coacervate droplet confined within a microfluidic channel and exposed to an electric field of 10 V/cm. FITC-labelled PEG is also added but cannot be detected. Movie is shown at 20 times of real time speed at 20 frames per second. Total duration of recording was 12 seconds in real time.

Movie S5. Confocal fluorescence microscopy video of the transition from a bilateral circulation to a quadrupole (Taylor) circulation in a coacervate droplet confined within a microfluidic channel and exposed to an electric field of 10 V/cm. Movie is shown at 20 times of real time speed at 20 frames per second. Total duration of recording was 15 seconds in real time.

Movie S6. Confocal fluorescence microscopy video of transition from a bilateral circulation to an asymmetric triple-pole circulation in a coacervate droplet confined within a microfluidic channel and exposed to an electric field of 10 V/cm. Movie is shown at 20 times of real time speed at 20 frames per second. Total duration of recording was 10 seconds in real time.

Movie S7: Confocal fluorescence microscopy video of the circulation coupled with vacuolization in a PLL (0.5 mg mL^{-1})/ss-oligo (0.75 mg mL^{-1}) droplet confined within a microfluidic channel and exposed to an electric field of 20 V/cm. The video showing the corresponding particle imaging velocimetry is also attached. Movie is shown at 5 times of real time speed at 5 frames per second. Total duration of recording was 10 seconds in real time.

Movie S8: Confocal fluorescence microscopy video of the circulation coupled with vacuolization in a PLL (2.0 mg mL^{-1})/ss-oligo (0.75 mg mL^{-1}) droplet confined within a microfluidic channel and exposed to an electric field of 10 V/cm. The video showing the corresponding particle imaging velocimetry is also attached. Movie is shown at 5 times of real time speed at 5 frames per second. Total duration of recording was 8 seconds in real time.

Movie S9: Confocal fluorescence microscopy video of the circulation coupled with vacuolization in a PLL (2.0 mg mL^{-1})/ss-oligo (3.0 mg mL^{-1}) droplet confined within a microfluidic channel and exposed to an electric field of 20 V/cm. The video showing the corresponding particle imaging velocimetry is also attached. Movie is shown at 10 times of real time speed at 10 frames per second. Total duration of recording was 11 seconds in real time.

Movie S10: Confocal fluorescence microscopy video of the movement of RITC-labelled HRP a PLL (0.5 mg mL^{-1})/ss-oligo (0.75 mg mL^{-1}) droplet confined within a microfluidic channel and exposed to an electric field of 10 V/cm. Movie is shown at 10 times of real time speed at 10 frames per second. Total duration of recording was 15 seconds in real time.

Movie S11: Confocal fluorescence microscopy video of the movement of RITC-labelled HRP a PLL (0.5 mg mL^{-1})/ss-oligo (0.75 mg mL^{-1}) droplet confined within a microfluidic channel and exposed to an electric field of 30 V/cm. Movie is shown at 10 times of real time speed at 10 frames per second. Total duration of recording was 11 seconds in real time.