Supporting Information for:

Sustained formation, movement and destruction of complex coacervates in a waste-less system driven by electricity

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Supplementary methods

Sample preparation for cyclic voltammetry (CV)

All samples were prepared using a background solution containing 50 mM of Tris buffer (pH = 7.4) and 100 mM of NaCl in deionized water. The concentration of polyelectrolytes for the CV experiments was reduced by half due to the larger sample volumes. The samples had the following concentrations: KKKC – 1.67 mM; pAA – 2 mM (in monomer units), MV – 3.34 mM. The formation of coacervates at this lower polyelectrolyte concentration was confirmed by optical microscopy. Coacervates were prepared from the mixture of 1.67 mM KKKC and 2 mM pAA in the background solution (15 ml) by oxidation (35 min, 0.9V, flat silver electrodes) in a glass vial upon stirring. CV measurements were performed directly after the oxidation to minimize the coalescence and sedimentation of the coacervates. After the analysis, MV was added to the mixture and the CV was repeated. To obtain the pAA+MV control curve, the same oxidative conditions were applied to a solution of pAA, after which MV was added to the solution directly before the CV measurement.

Cyclic voltammetry (CV)

CV was performed using a PARSTAT 2273 potentiostat with a silver electrode (OD x ID = 6.0 x 3.0 mm, A 002419, ALS) or a Glassy Carbon Electrode – GCE (OD x ID = 6.0 x 3.0 mm, A002012, ALS) as a working electrode, Pt wire as a counter electrode and a Ag/AgCl (Re-1S Ag/AgCl in 3 M NaCl – length: 50 mm – OD: 4.5 mm, A-012168, ALS) reference electrode. The scans were recorded from -0.9 to 0.2 V for the silver electrode and from -1 to 1 V for the GCE with a 100 mV/s scan rate. The measurements were performed under nitrogen. Typically, 10 CV cycles were performed to increase the intensity of the anodic and cathodic peaks of KKKC.

Ellman's test for the detection of thiols

UV-VIS analysis was performed on a PerkinElmer Lambda 650 S UV/Vis spectrometer. Spectra were recorded from 600 to 300 nm with a 1 nm interval and a 266.75 nm/min scan speed against air as a reference.

All samples contained 50 mM Tris buffer (pH = 7.4) and 100 mM NaCl.

To obtain electrochemically-oxidized coacervates, 1 ml of a typical 2:5:1 KKKC/pAA mixture was oxidized (0.9 V) while stirring for 30 min in a 2 ml microtube, equipped with two flat silver electrodes.

Chemical oxidation of a KKKC/pAA mixture was performed by adding 2 eq. of $NaBO_3$ (20 mM, freshly prepared solution) to an already diluted sample of 2:5:1 KKKC/pAA and letting it equilibrate for 1 hour.

Solutions of KKKC, KKKC/pAA, chemically oxidized $(KKKC)_2$/pAA coacervates, and coacervates freshly-prepared by electrochemical oxidation were freshly prepared as indicated. An aliquot of each sample was diluted to a final concentration of 0.2 mM of KKKC. Ellman's reagent (10 mM suspension of 5,5-dithio-bis-(2-nitrobenzoic acid), or DNTB, stabilized with 2 mM EDTA) was added to each sample to a final concentration of DNTB of 0.5 mM. A UV-Vis spectrum was recorded after a 5 min incubation. The blank solution contained 50 mM Tris buffer (pH = 7.4) and 100 mM NaCl.

Nuclear Magnetic resonance (NMR)

^{1}H-NMR was performed using a 300 MHz Avance III HD (Bruker) spectrometer, with D_2O as a solvent. Chemical shifts were adjusted using the peak of the solvent as a reference (4.79 ppm), and different spectra were aligned based on the peak of the Tris buffer (3.75 ppm).

All stock solutions (100 mM pAA, 200 mM Tris buffer (pH = 7.4)) and samples were prepared in D_2O. All samples contained 50 mM Tris buffer and 100 mM NaCl (before oxidation). KKKC, NaCl and $NaBO_3$ were added to the samples as solids. The initial concentration of KKKC in all samples was 3.34 mM, the concentration of pAA was 4 mM (in momoner units), matching the typical 2.5:1 KKKC:pAA system. First, a KKKC control and a KKKC/pAA mixture (1 ml each) were prepared and analyzed by ^{1}H-NMR. The KKKC/pAA mixture was then transferred into a 2 ml microtube with a stirring bar, equipped with flat silver electrodes. The mixture was oxidized (0.9 V) while stirring for 90 min. The obtained coacervates were dissolved by adding solid NaCl to a final concentration of 2 M, after which the solution was analyzed by ^{1}H-NMR. To prepare the oxidation controls, fresh KKKC solutions were either electrochemically oxidized in the same conditions or chemically oxidized with $NaBO_3$ (3.7 equiv., approximately 1 hour before recording the spectrum). Both controls contained 2 M of NaCl.

Fluorescence microscopy

Fluorescent microscopy was performed on a Nikon Eclipse Ts2 microscope Type 120c in fluorescent mode.

Stock solutions of all dyes were prepared in water to a 20 μM concentration. To increase the solubility of fluorescein, NaOH (20 μM) was added to the stock solution.

Polyelectrolyte mixtures 1:1 KKKC:pAA, 2.5:1 KKKC:pAA, 1:1 KKKC:ATP and 2.5:1 KKKC:ATP were prepared as indicated in the main text. Additionally, 2.5:1 KKKC:pAA mixtures were prepared with [NaCl] = 50 and 10 mM.

Each sample (200 μL) was oxidized with a potential of 0.7 V in the standard undivided cell. The time of oxidation was 20 min in the case of pAA-containing samples and 100 min for the samples with ATP. After coacervate formation, each sample was split in four parts and transferred to new wells. An aliquot of each dye (2 μL) was added to every well and mixed via repeated pipetting. The well plate was covered and the mixtures were left to equilibrate overnight. Fluorescence microscopy images were recorded using a 455 nm excitation wavelength for fluoresceine and Brooker's merocyanine, and a 525 nm excitation wavelength for Rhodamine B and Nile red.

Supplementary Figures

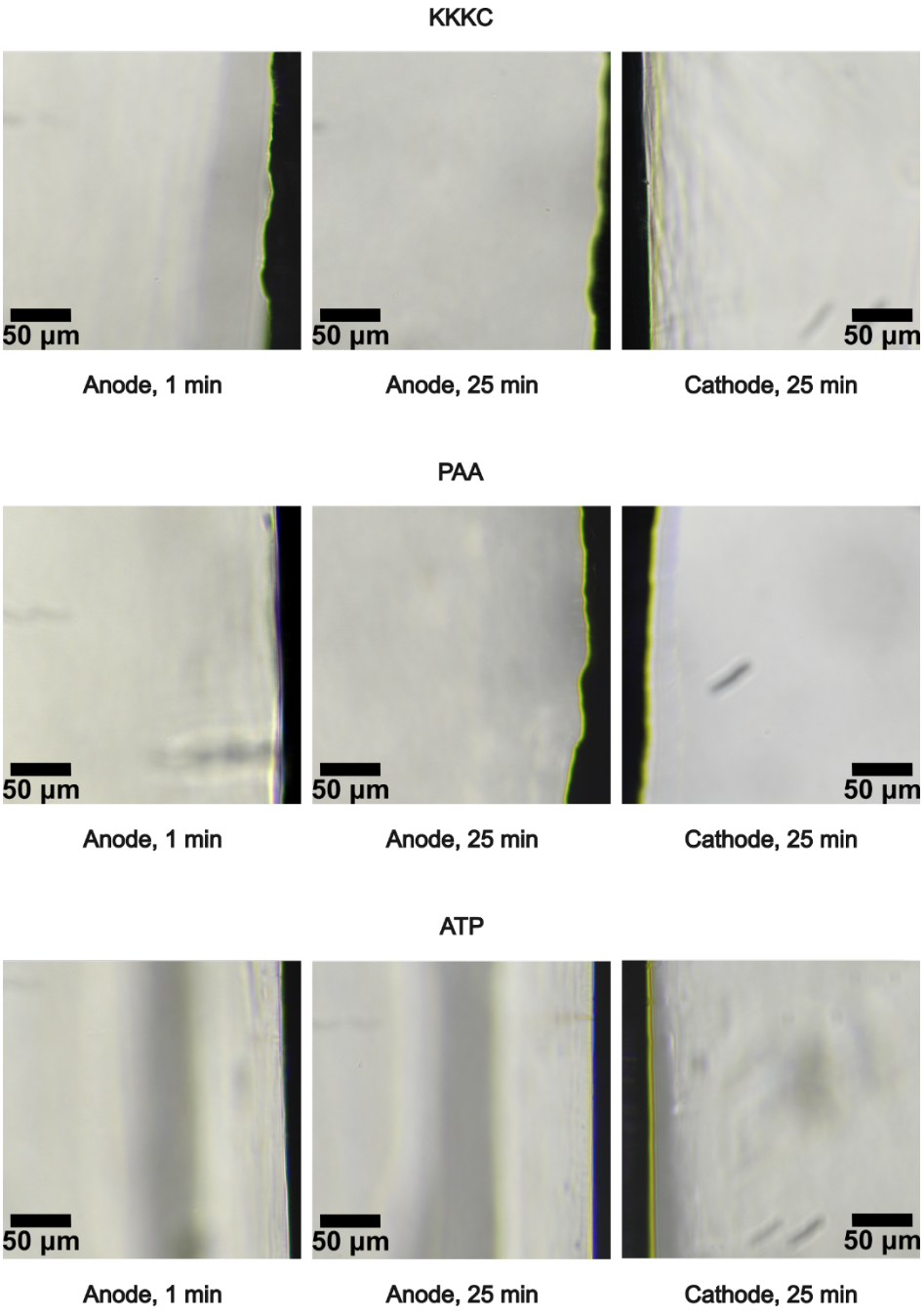

Figure S1. Microscope images of the area next to the electrodes showing the absence of the coacervate formation upon application of an electric potential (0.7 V) in the following control solutions: KKKC (1.34 mM in 10 mM NaCl), pAA (4 mM in 100 mM NaCl), ATP (1.34 mM in 10 mM NaCl). All solutions contained 50 mM Tris buffer (pH = 7.4).

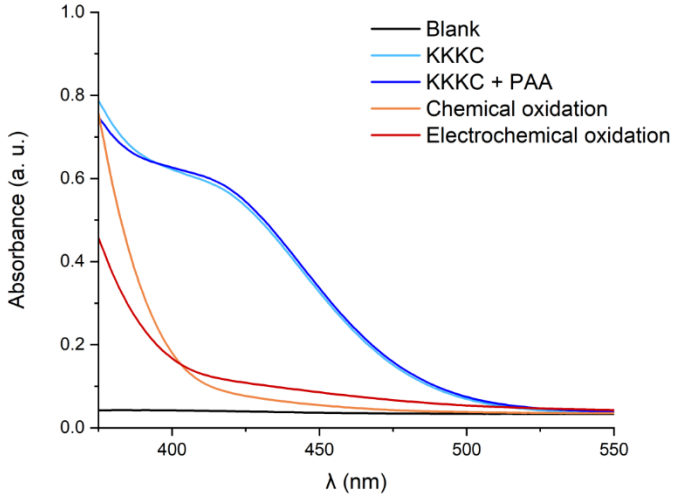

Figure S2. Ellman's test for the UV-Vis detection of thiols. Solutions of KKKC and KKKC + pAA show a band at 412 nm, characteristic to the reaction between free thiols and DNTB (5,5′-dithiobis-(2-nitrobenzoic acid)). No thiols are detected in the chemically or electrochemically oxidized KKKC + pAA samples, indicating that all thiol groups had been completely oxidized. [KKKC] = 0.2 mM, [pAA] = 0.08 mM (in charge units). All samples contained 50 mM Tris buffer (pH = 7.4), 100 mM NaCl, and 0.5 mM of DNTB. The chemically oxidized sample contained 2 equiv. of $NaBO_3$, and the electrochemically oxidized sample was preparing by applying a 0.9 V potential for 30 minutes to a KKKC/pAA mixture.

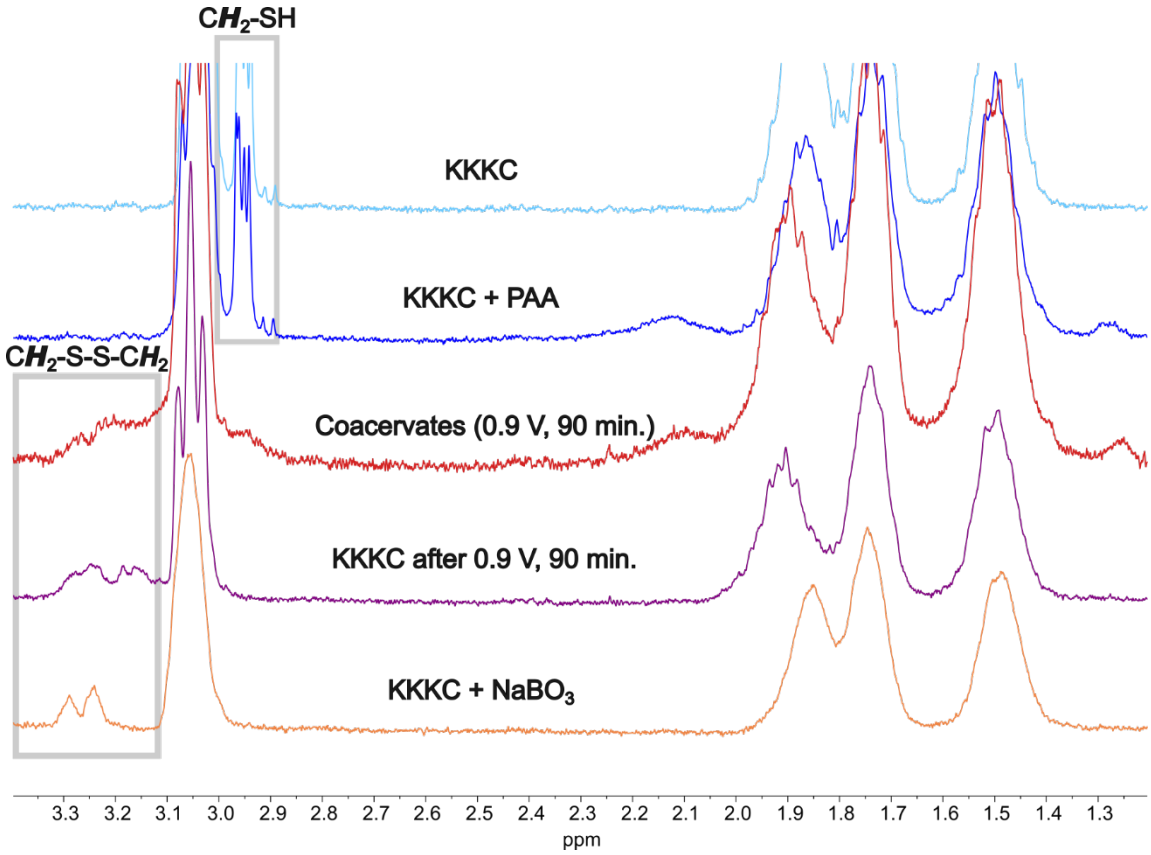

Figure S3. ^{1}H-NMR spectra (300 MHz, D_2O) of fresh KKKC solution, polyelectrolyte mixture, coacervates prepared by electrochemical oxidation and controls with electrochemically and chemically oxidized KKKC. The signals corresponding to the CH_2 protons next to the thiol/disulfide group are highlighted. The signals at 2.2-2.1 ppm and 1.3-1.2 ppm correspond to the protons of pAA. The signals between 2.0 and 1.4 ppm and at 3.1-3.0 ppm correspond to the protons of the lysine side-chains.

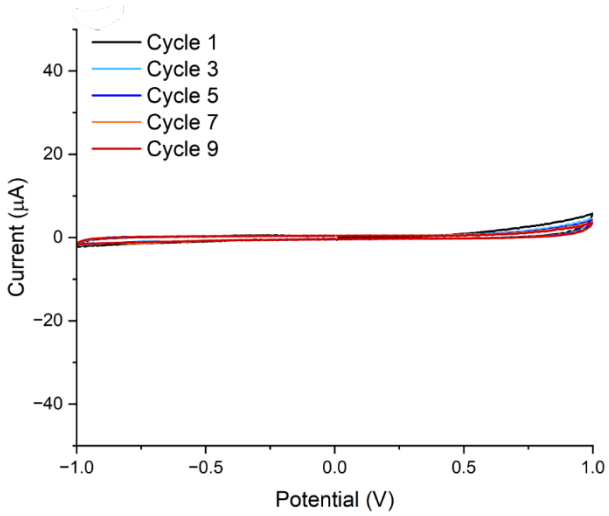

Figure S4. Cyclic voltammetry curve of KKKC, recorded with a GCE working electrode (vs Ag/AgCl). No electrochemical response could be detected within the working window of the electrode. [KKKC] = 1.67 mM. The solution contains Tris buffer (50 mM) and NaCl (100 mM).

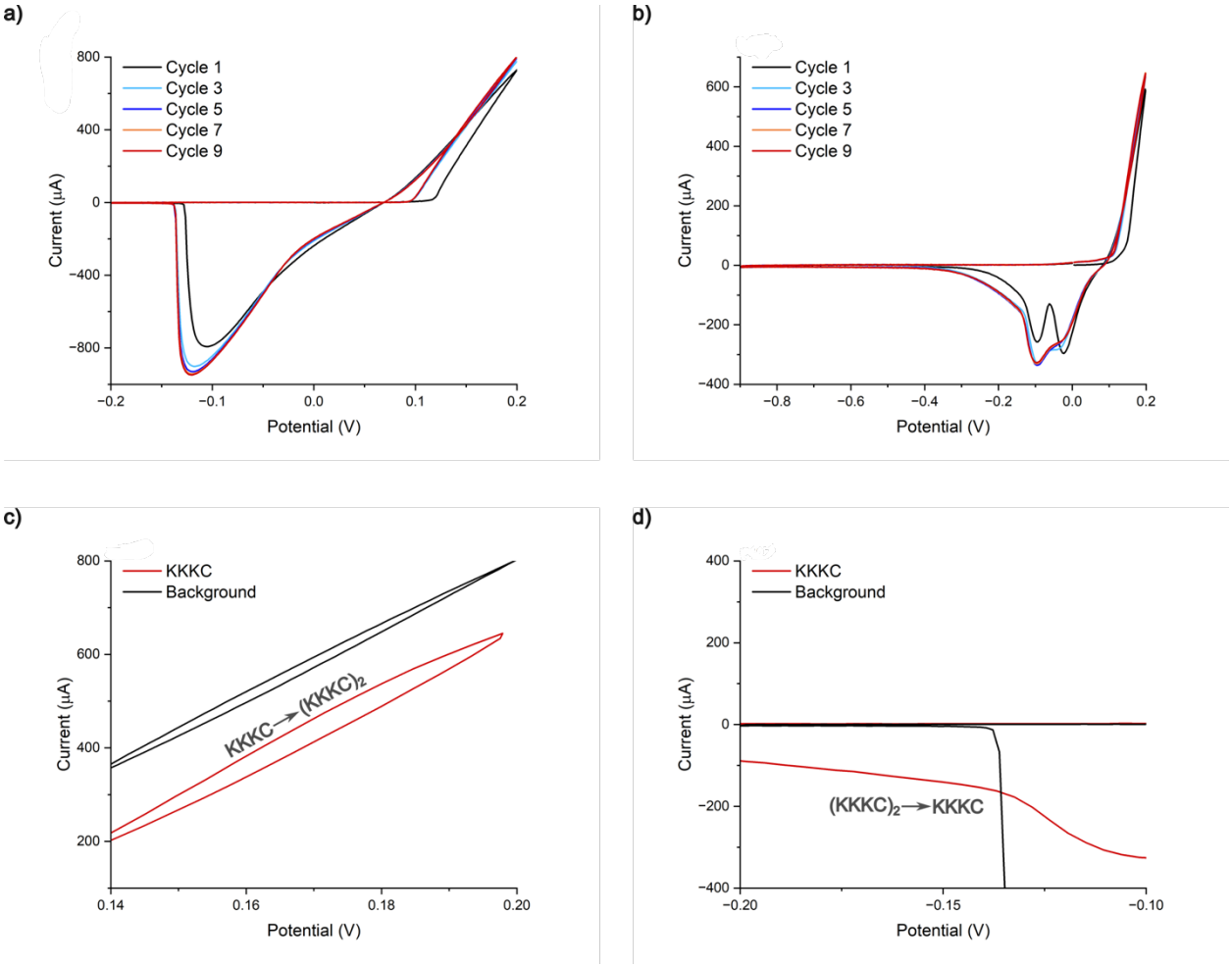

Figure S5. Cyclic voltammetry study of KKKC using a silver working electrode (vs Ag/AgCl). a) CV curves of the background electrolyte, showing the anodic oxidation of silver (> 0.1 V) and a cathodic peak corresponding to the reduction of trace amounts of dissolved oxygen. b) CV curves of KKKC in the same conditions. In addition to the signals that were already present in the background CV, two additional broad peaks can be observed: a new oxidation band between 0.14 and 0.20 V, and a reduction band between -0.1 and -0.3 V. c) Comparison between the CV curves of background electrolyte and KKKC (Cycle 9), zoomed in to the region corresponding to anodic oxidation. d) Comparison between the CV curves of background electrolyte and KKKC (Cycle 9), zoomed in the region corresponding to cathodic reduction. [KKKC] = 1.67 mM. Both solutions contain Tris buffer (50 mM) and NaCl (100 mM).

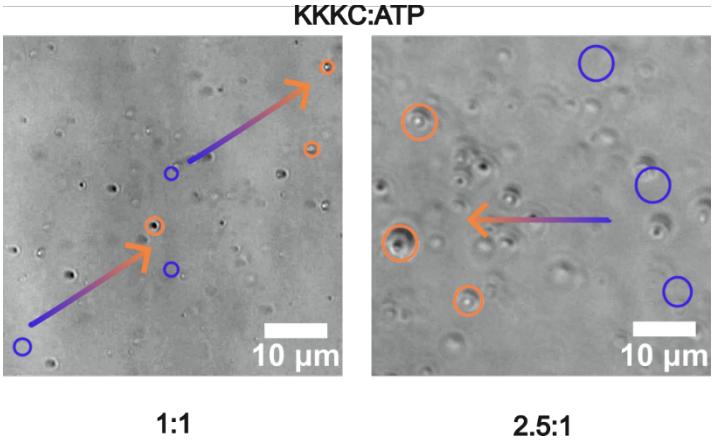

Figure S6. Electrophoretic movement of KKKC/ATP coacervates at different mixing ratios. The numbers indicated in the images represent the mixing ratio between KKKC and ATP in charge units. [KKKC] = 1.34 mM or 3.34 mM, [ATP] = 1.34 mM. All samples contained Tris buffer (50 mM) and NaCl (10 mM). The strength of the electric field was 0.95 V/cm. The scale bars are 10 µm

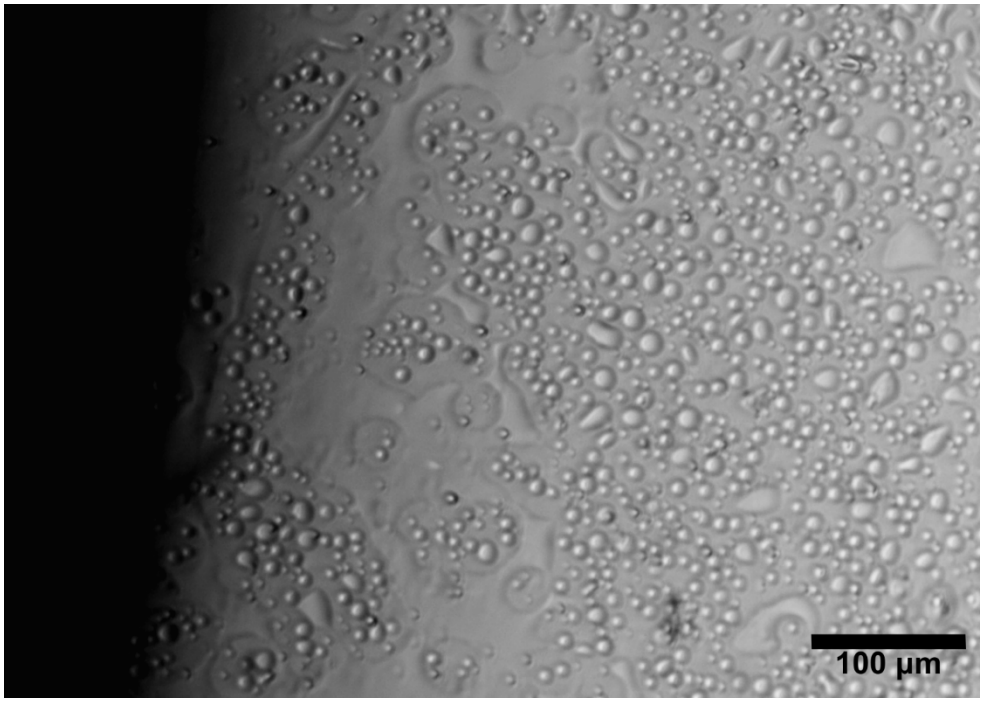

Figure S7. Micrograph of the bottom of the cell next to the cathode, ~20 minutes after the application of a 0.7 V potential. The accumulation of a continuous coacervate phase radiating from the electrode indicates that electrochemical reduction of $(KKKC)_2$ contained in the coacervate droplets is slower than their coalescence and sinking. [KKKC] = 3.34 mM, [pAA] = 4 mM (in monomer units). The ratio between positive and negative charges was 2.5:1. The scale bar is 100 µm

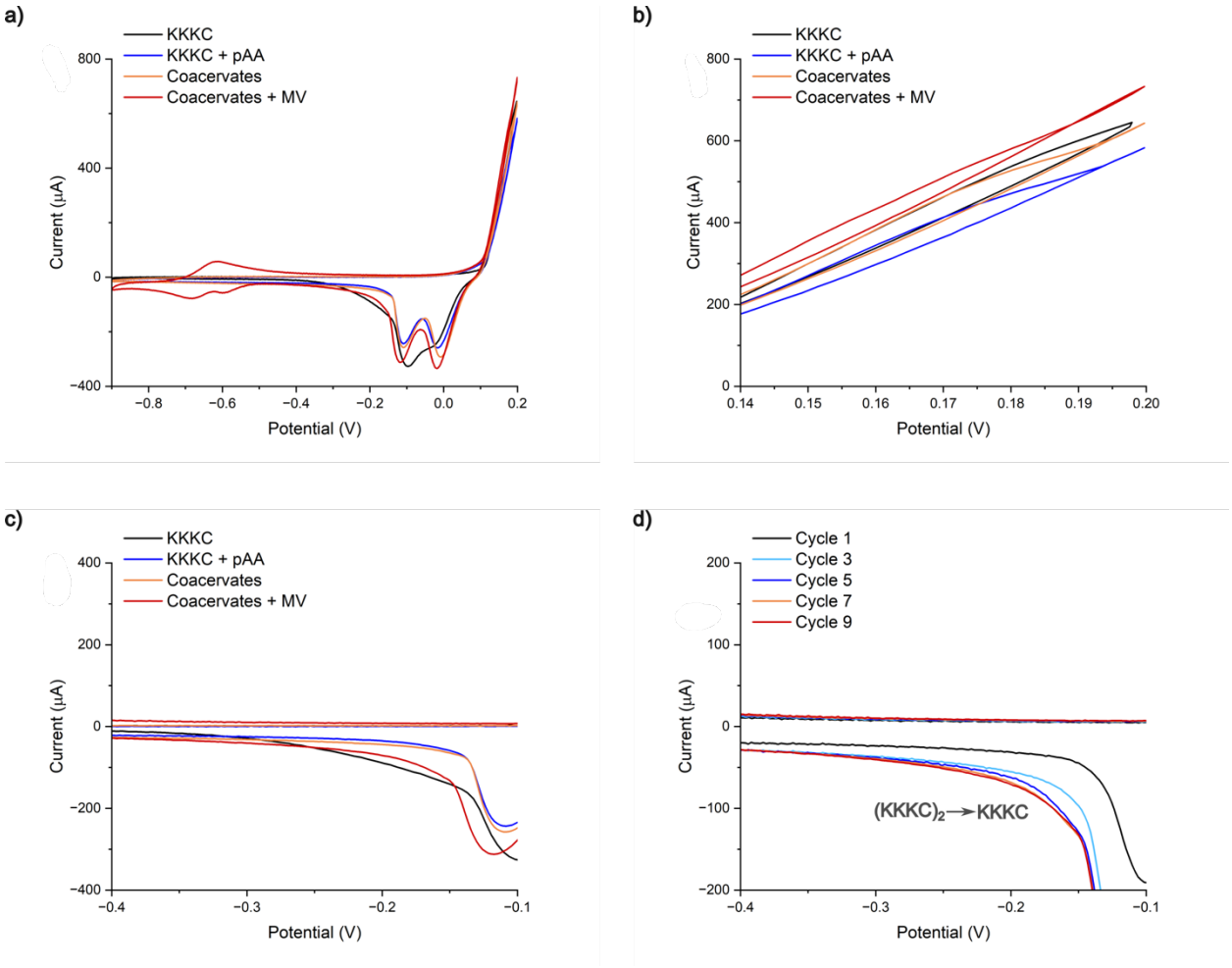

Figure S8. CV curves (Cycle 9) of KKKC in presence of pAA and MV. a) Full CV curves. b) Region corresponding to KKKC oxidation. The amplitude of the peak overlapping with silver oxidation is not significatively different between different samples. c) Region corresponding to the reduction of $(KKKC)_2$. The current of the $(KKKC)_2 \rightarrow$KKKC band is reduced in the samples containing pAA, and restored when MV is added. d) Consecutive CV cycles in a sample containing coacervates and MV. We hypothesize that several cycles of oxidation / reduction mediated by MV dissolve the coacervate in the surface of the electrode, releasing free $(KKKC)_2$. [KKKC] = 1.67 mM. [pAA] = 2 mM in monomer units. All solutions contain Tris buffer (50 mM) and NaCl (100 mM).

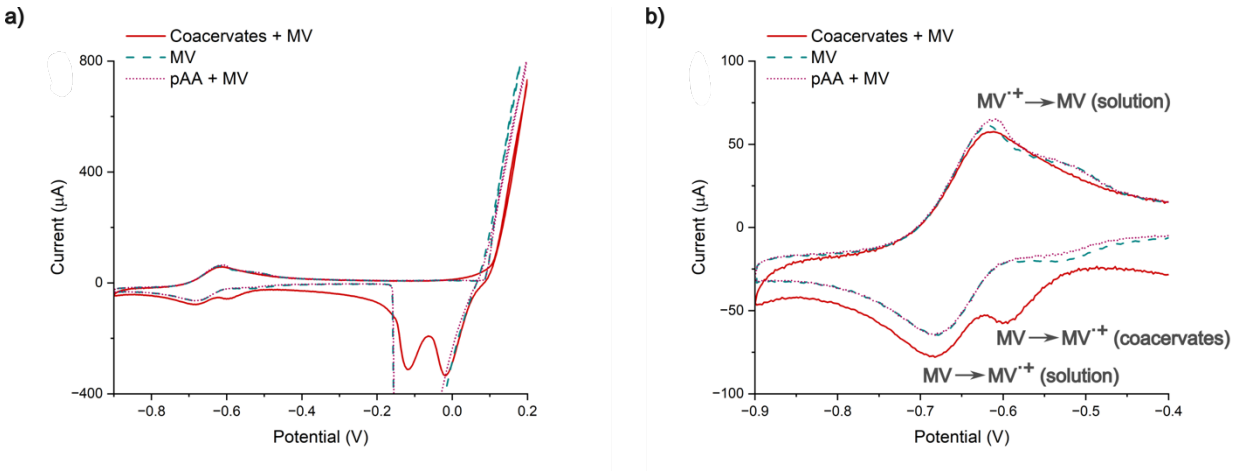

Figure S9. CV curves (Cycle 9) of MV in samples containing either coacervates or pAA. a) Full CV curves. b) Region corresponding to MV oxidation and reduction. The apparently irreversible cathodic band at -0.6 V appears only in the sample containing coacervates, supporting the hypothesis that it corresponds to the oxidation of MV dissolved in the coacervate phase. [KKKC] = 1.67 mM. [pAA] = 2 mM in monomer units, [MV] = 3.34 mM. All solutions contain Tris buffer (50 mM) and NaCl (100 mM).

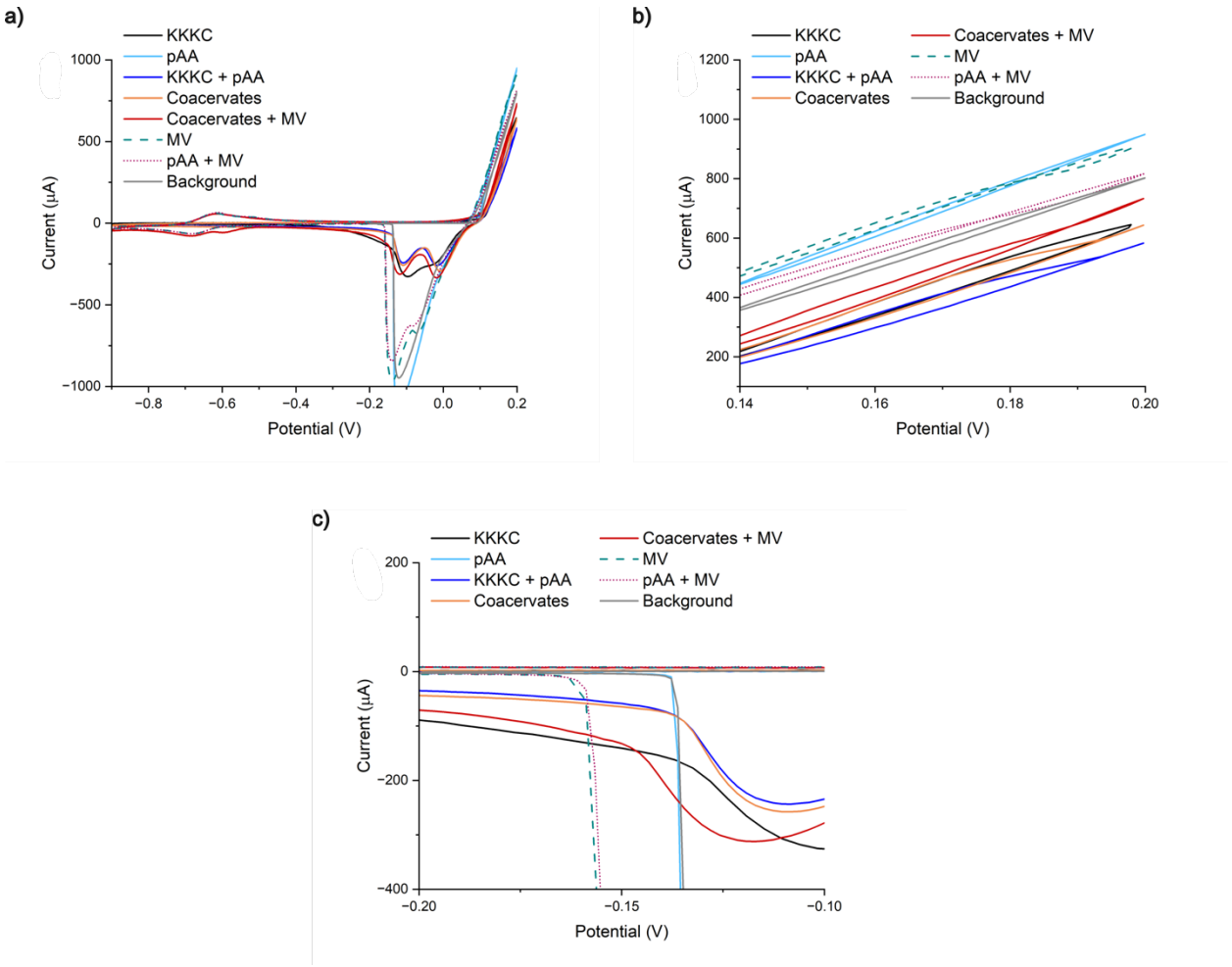

Figure S10. Comparison of the CV curves (Cycle 9) of all samples in this study. a) Full CV curves. b) Region corresponding to KKKC oxidation. c) Region corresponding to $(KKKC)_2$ reduction. The concentrations and conditions for each experiment are indicated in the Supplementary Methods.

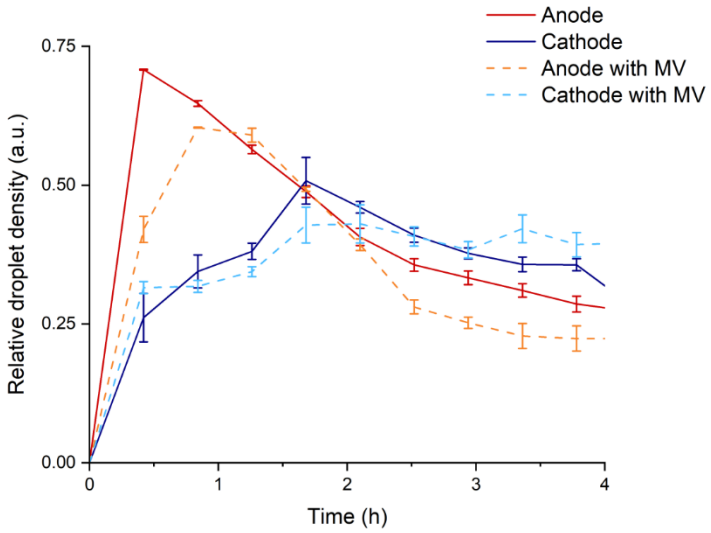

Figure S11. Relative droplet density in 2.5:1 KKKC:pAA samples in presence and absence of MV, upon continuous application of an electric potential (0.7 V). The proximity of the anode and the cathode were imaged separately, and the droplet density was calculated from the average grey value of different areas of the image (see Supplementary Methods). After the first 4 h, the values for all systems reach a plateau, where the gray value is predominantly affected by a layer of coalesced droplets on the background, and the impact of individual droplets being formed and moving is negligible. After this time, the number of droplets was counted manually (see Figure 5 in the Main Text).

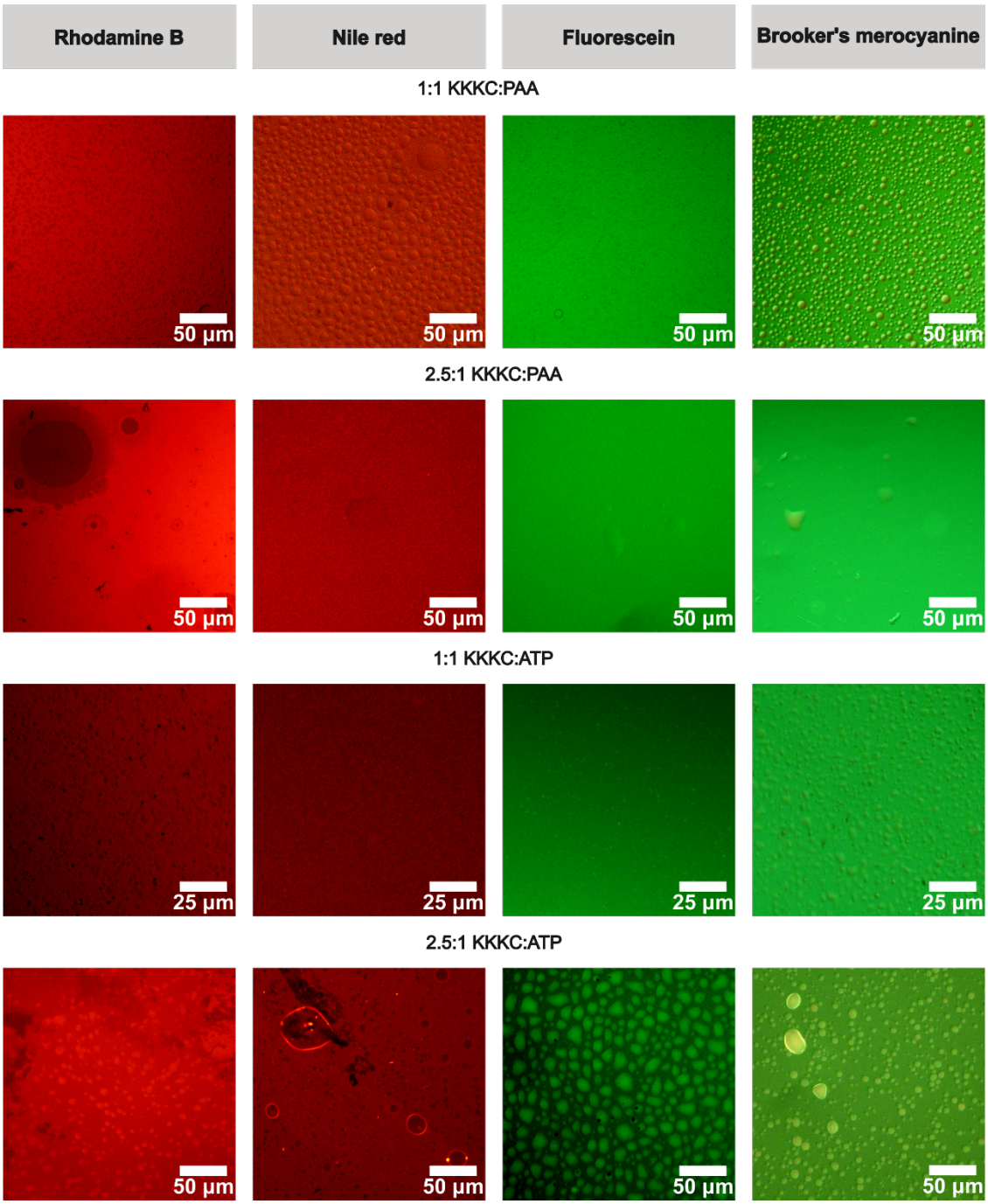

Figure S12. Fluorescence microscopy images for KKKC/pAA and KKKC/ATP coacervates in presence of neutral (Nile Red, Brooker's merocyanine), and charged (fluorescein and Rhodamine B) dyes. The partitioning of different dyes depends on the chemical composition and mixing ratio of the coacervates. All samples contained NaCl (100 mM) and Tris buffer (50 mM). Coacervates were generated by electrochemical oxidation of a sample with [KKKC] = 1.34 mM or 3.34 mM, [pAA] = 4 mM and [ATP] = 1.34 mM in monomer units. The concentration of all dyes was 0.8 µM. Dyes and coacervates were incubated overnight before imaging.

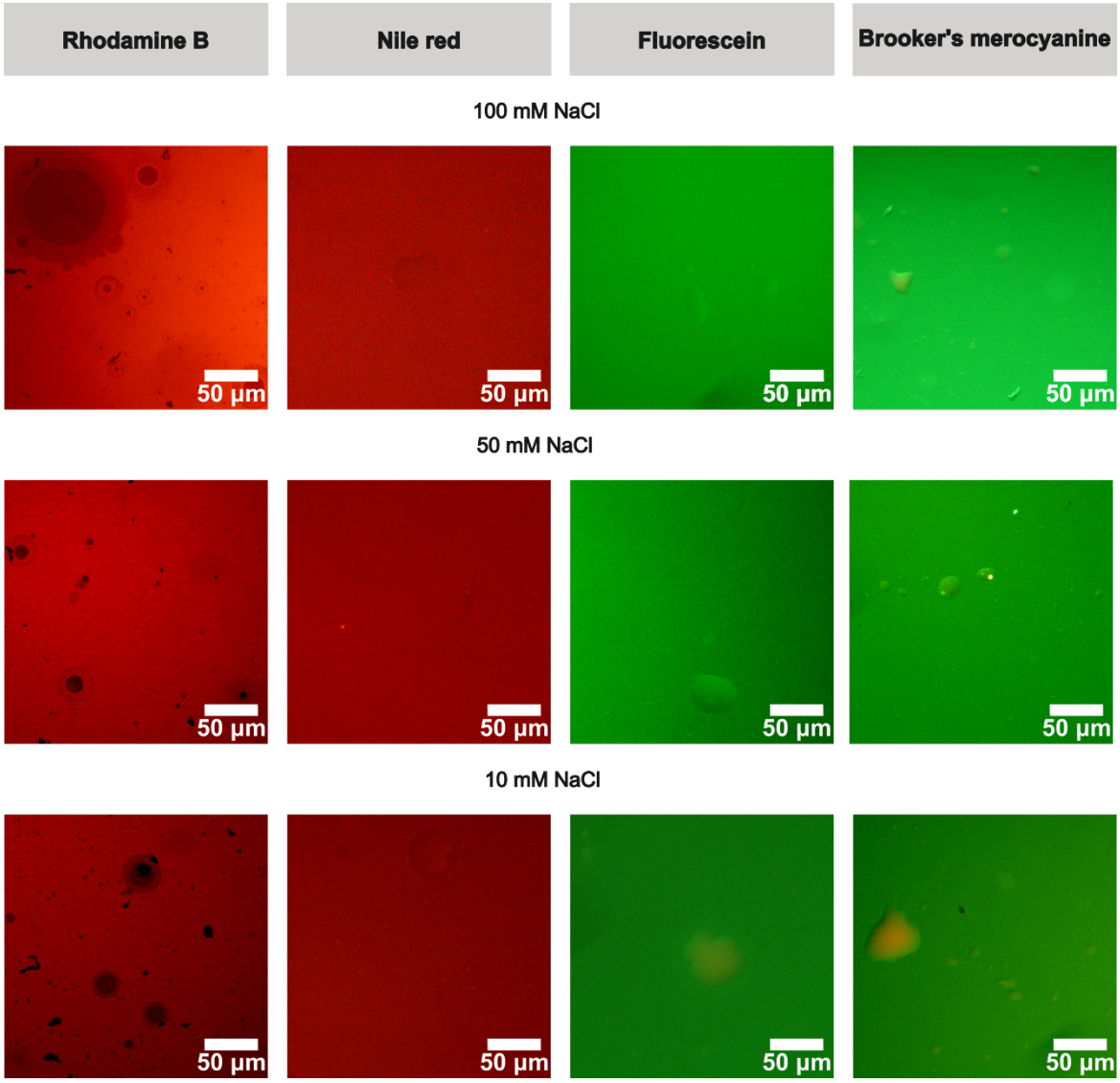

Figure S13. Fluorescence microscopy images of different dyes into 2.5:1 KKKC:pAA coacervates at different salt concentrations. While the encapsulation of Rhodamine B and Nile red is not affected by the concentration of NaCl, the partition of fluoresceine and Brooker's merocyanine into coacervate droplets increases as the ionic strength of the mixture decreases. All samples contained Tris buffer (50 mM). Coacervates were generated by electrochemical oxidation of a sample with [KKKC] = 3.34 mM and [pAA] = 4 mM in monomer units. The concentration of all dyes was 0.8 µM. Dyes and coacervates were incubated overnight before imaging.