



Supplementary Materials for

Blood-catalyzed n-doped polymers for reversible optical neural control

Sanket Samal *et al.*

Corresponding author: Krishna Jayant, kjayant@purdue.edu, Jianguo Mei, jgmei@purdue.edu

Science **392**, eadu5500 (2026)
DOI: 10.1126/science.adu5500

The PDF file includes:

Materials and Methods
Figs. S1 to S76
References

Other Supplementary Material for this manuscript includes the following:

MDAR Reproducibility Checklist

Materials and Methods:

Materials: All reagents for the synthesis and analysis were purchased from Sigma Aldrich, Alfa Aesar, Acros Organics, Oakwood Chemical, AmBeed, and TCI Chemicals and used without further purification unless otherwise mentioned. Solvents were purchased from Fisher Scientific and used without any further purification. BDF precursor acid (2,5-dihydroxy-1,4-benzenediacetic acid) was purchased from Santa Cruz Biotechnology and was recrystallized twice in water under nitrogen. All lyophilized hemoproteins (Hemoglobin, Myoglobin, and Cytochrome c) were purchased from Sigma.

UV-Vis-NIR Absorption Spectroscopy: UV-Vis-NIR absorption spectra were measured with an Agilent Technologies (Cary 5000/6000i) Cary Win UV-Vis-NIR spectrophotometer. Solution UV-Vis-NIR spectra were recorded in a quartz cuvette with a 1 cm path length. UV-Vis-NIR absorption spectra for RBCs/Blood samples were measured with a Thermo Scientific Genesys 30 Visible spectrophotometer. RBCs/Blood solution UV-Vis-NIR spectra were recorded in a polystyrene disposable cuvette with a 1 cm path length.

Fourier Transformed Infrared (FTIR) Spectroscopy: FTIR spectra were measured with a Thermo Nicolet 6700 FTIR spectrophotometer. The unpurified polymer solutions synthesized with Hemoglobin (Hb) were added to the diamond ATR, and measurements were obtained under ambient conditions.

Electron paramagnetic resonance (EPR): EPR measurements were performed on a Bruker EMX EPR Spectrometer. After preparing the samples in an oven-dried EPR tube, the sample was carefully frozen with liquid nitrogen. Next, the frozen sample was loaded into the pre-cooled sample cavity of the spectrometer with a helium cooling system for low-temperature experiments at 20 K. (Freq = 9.4799891 GHz; microwave power = 2.0 mW; mod. amp. = 2.0 G; time constant = 20.48 msec). The EPR signal intensity was determined by calculating the double integration.

Zeta-potential (ZP): The zeta potential of the n-PBDF solutions (100-fold dilution) was measured by determining electrophoretic mobility with a Malvern Nano-Z Zeta potential instrument (Malvern Ltd., UK). A 0.15 ml solution was loaded into a folded capillary cell (DTS1070), and measurements were conducted at 25 °C using a He-Ne laser light source at 633 nm with a maximum power of 4 mW. Each sample was measured three times, and the average value was reported as the final result.

Dynamic light scattering (DLS): DLS measurements of the n-PBDF solutions (100-fold dilution) were performed with a Wyatt DynaPro NanoStar II (Wyatt Tech, USA). A 45 μL solution was loaded into a quartz microcuvette. Measurements were conducted at 25 $^{\circ}\text{C}$ using a 120mW air-launched laser light source at 662 nm, and the intensity of light scattered at an angle of 90° was detected with a solid-state single-photon counting module. Data were acquired with an acquisition time of 10s and 10 acquisitions per measurement. Each sample was measured three times, and the average value was reported as the final result.

Raman spectroscopy: A 532 nm single longitudinal mode laser (CNI Laser) with a maximum output power of 200 mW is used as an excitation source. The laser is focused onto the sample using a 63X air objective lens (Leica, HI PLAN 63x/0,75) with an incident power of 60 mW. The Raman signal is collected in the reflection direction. The spRS spectral acquisition is carried out using a Kymera 328i spectrograph paired with an EMCCD (Newton 920, 1024×256 pixels, Oxford Instruments), which is cooled to -80°C to minimize thermal noise during acquisition. To block the 532 nm excitation laser effectively, a long pass filter is placed before the 80 μm spectrometer entrance slit. Signal dispersion is accomplished using a 600 lines/mm grating within the spectrometer. The spectral acquisition process is achieved through Solis software (Oxford Instruments).

Cyclic Voltammetry (CV): CV measurements were carried out using a Shanghai Chenhua Instrument CHI760E potentiostat with a three-electrode setup. Redox properties of the unpurified drop-cast n-PBDF (Hb) films (in 0.2M tetrabutylammonium bis-trifluoromethanesulfonimide (TBA-TFSI) electrolyte dissolved in propylene carbonate) were tested in a three-electrode cell using an ITO/n-PBDF (Hb) working electrode, an Ag/AgCl reference electrode, and a Pt wire counter electrode. Cyclic voltammetry was performed at a scan rate of 20 mV s^{-1} , unless specified otherwise. All measurements were performed under ambient conditions.

Spectro-Electrochemistry: Spectro-electrochemical measurements were performed by fitting the CV setup inside a quartz cuvette in the UV–Vis–NIR absorption spectrophotometer. Potentials were applied for 20s before recording the UV–Vis–NIR absorption spectrum at each voltage to ensure stabilization of the optical trace. The unpurified drop-cast n-PBDF (Hb) films on ITOs (in 0.2M tetrabutylammonium bis-trifluoromethanesulfonimide (TBA-TFSI) electrolyte dissolved in propylene carbonate) were tested in a cuvette of 1 mm path length. The same Ag/AgCl reference

electrode used for the CV was used in these experiments. Potential steps of 0.2 V were then used to evaluate the electrochromic behavior of the polymer.

Conductivity and Sheet Resistance Measurements: Sheet resistance was measured through the top-contact four-probe measurement in the Filmetrics R50-200-4PP Resistance Mapper. To obtain uniform drop-casted thin-film samples, the polymer solutions were sonicated in an ultrasonic bath for 5 min and then stirred for 2 hours at room temperature. Before drop casting, all polymer samples were treated by a vortex mixer for 5 mins. Polymer solutions (200 μ L) were deposited on the clean Si/SiO₂ substrates and were then placed in a vacuum oven at room temperature for at least 12 hours for the complete removal of the residual solvent. After that, the thin films were annealed at 80 °C for 15 mins under a vacuum and then kept under nitrogen flow until the substrates cooled down to room temperature. The conductivity of the films was then calculated by the equation below:

$$\sigma = \frac{1}{R_s t}$$

where R_s is the measured sheet resistance, and t is the film thickness (measured by a profilometer). A minimum of three replicates were measured for consistent results.

Hemolysis Assay: Hemolysis assays were based on a previously described method (67). Human Female Whole Blood was purchased from BioIVT and used before its expiration date. 100 μ L of blood was aliquoted into a 1.5 mL Eppendorf tube, and 500 μ L of sterile 0.9% NaCl was added. Tubes were gently inverted to mix and then centrifuged at 500Xg for 7 minutes. The supernatant was carefully removed, and the pellet was washed 2X with 500 μ L of 0.9% NaCl. The pellet was then resuspended in 800 μ L of Red Blood Cell (RBC) buffer (10 mM Na₂HPO₄, 150 mM NaCl, 1 mM MgCl₂, pH 7.4). To evaluate the hemolytic activity of compounds, they were added at desired concentrations (3% DMSO final) and transferred to a 96 U-well plate well. Negative control wells contained 4 μ L of DMSO, and positive controls contained 4 μ L of 30% Triton X-100. In each well, 76 μ L of RBC buffer and 40 μ L of the resuspended red blood cells were added. This was incubated for 1h at 37 °C. The plate was then spun at 500Xg for 5 min, and the supernatants from each sample (75 μ L) were transferred to a flat-well 96-well plate. The absorbance of these supernatants at 540 nm was then measured using a SpectraMax iD3 plate reader. Percent hemolysis was calculated

relative to the average absorbance values for the positive and negative controls. A minimum of three biological replicates were performed.

SRB Cytotoxicity: Cytotoxicity assays were based on a previously described method (68). A549 non-small cell lung cancer cells (ATCC CCL-185) were obtained directly from ATCC and used within 20 passages. A549 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U mL⁻¹ penicillin, and 100 µg mL⁻¹ streptomycin. For cytotoxicity testing, cells were seeded at 20,000 cells per well and allowed to adhere overnight. Cells were then treated with the compound at desired concentrations (1% DMSO final) or vehicle control for 72 hours at 37 °C. Viability was assessed utilizing Sulfurhodamine B after a 1-hour incubation period. Absorbance was measured using a SpectraMax iD3 plate reader (@510 nm). The percent death was calculated by subtracting the background from all wells and setting 0% death to controls.

SRB Pulse Cytotoxicity: A549 non-small cell lung cancer cells (ATCC CCL-185) were obtained directly from ATCC and used within 20 passages. A549 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U mL⁻¹ penicillin, and 100 µg mL⁻¹ streptomycin. For pulse cytotoxicity testing, cells were seeded at 20,000 cells per well and allowed to adhere overnight. Cells were then treated with the compound at the desired concentration (1% DMSO final) or vehicle control. After a 6-hour incubation period at 37 °C, the cells were washed with warmed phosphate-buffered saline (PBS) three times and incubated for 24 hours in RPMI media with 10% fetal bovine serum, 100 U mL⁻¹ penicillin, and 100 µg mL⁻¹ streptomycin. Cell viability was assessed utilizing Sulfurhodamine B after a 1-hour incubation period. Absorbance was measured using a SpectraMax iD3 plate reader (@510 nm). The percent death was calculated by subtracting the background from all wells and setting 0% death to controls.

Cell viability assay in tissue: The skin tissue at the subcutaneous injection site was collected, embedded in 4% agarose, and sectioned (500µm) using a vibratome. The sectioned tissue was incubated in 2µM Calcein-AM and 4µM Ethidium homodimer-1 (in PBS) at 37 °C for 30 minutes before being washed with PBS and imaging under a confocal microscope.

Toxicity in Mice: Animals were subjected to subcutaneous injection of 0.1mL of a 10mM concentrated solution for each of the three different formulations: **i)** Monomer (BDF) mixed with 1wt% surfactant (TPGS-750-M) in PBS, **ii)** Aqueous polymeric n-PBDF solution (pre-synthesized *in vitro* with BDF and Hemoglobin in 1wt% TPGS-750-M in PBS), and **iii)** Monomer (BDF)

mixed with Hemoglobin in 1wt% TPGS-750-M in PBS. For each formulation, solutions were injected into 5 mice, where 1 was left as control, three were humanely euthanized, and blood was collected at 24 hours, 3 days, and 7 days to look at complete blood count (CBC/Chemistries) to determine toxic effects, and the last one was left as a reserve. Each mouse was closely monitored every 15 minutes for the first two hours and then hourly for up to 4 hours for any signs of distress or fall. After that, they were checked every 4 hours until they were humanely euthanized, except during the nighttime. Purdue Veterinary Clinical Pathology Laboratory processed the blood work, and the results were generated using the Siemens Advia 2120i Hematology analyzer.

Acute slice preparation: All animal-related experiment procedures were conducted in accordance with the guidelines of the Purdue Institutional Animal Care and Use Committee (IACUC) (protocol number: 1910001968). Male and female C57BL/6J mice (Jackson Laboratory, 000664, 12 to 24 weeks old) were used for acute brain slices preparation. The animals were anesthetized with isoflurane and trans-cardiac perfused with ice-cold NMDG solution (92mM NMDG, 30mM NaHCO₃, 1.2mM NaH₂PO₄, 20mM HEPES, 2.5mM KCl, 25mM glucose, 5mM sodium ascorbate, 3mM sodium pyruvate, 2mM thiourea, 0.5mM CaCl₂, 10mM MgCl₂, 5mM N-acetyl-L-cysteine, pH adjusted to 7.3 with HCl, osmolarity 300 to 310mOsm). All physiological buffers were continuously bubbled with carbogen (95% oxygen and 5% CO₂) during the slicing and patch-clamp recording procedure. The brain was then extracted and sliced into 350µm coronal slices in ice-cold NMDG solution with a vibratome (Leica VT1200S). The brain slices were recovered in 34°C NMDG solution for 5 to 10 minutes before being transferred into room temperature HEPES artificial cerebrospinal fluid (ACSF) (92mM NaCl, 2.5mM KCl, 1.25mM NaH₂PO₄, 30mM NaHCO₃, 20mM HEPES, 25mM glucose, 5mM sodium ascorbate, 3mM sodium pyruvate, 2mM thiourea, 2mM CaCl₂, 2mM MgCl₂, 5mM N-acetyl-L-cysteine, pH 7.3, osmolarity 300 to 310mOsm) for at least one hour before recording.

Patch clamp electrophysiology: Patch clamp recording was performed at 34°C recording ACSF (125mM NaCl, 3mM KCl, 25mM NaHCO₃, 1.25mM NaH₂PO₄, 25mM glucose, 3mM sodium pyruvate, 1mM sodium ascorbate, 1.3mM CaCl₂, 1mM MgCl₂, pH 7.4, osmolarity 300 to 310mOsm) with 4 to 6 MΩ borosilicate pipettes (or 10 to 15 MΩ pipettes for dendritic recordings) pulled with a pipette puller (Sutter Instruments P1000). The patch pipette is filled with internal solution (130mM potassium gluconate, 7mM KCl, 10mM HEPES, 5mM NaCl, 35mM sucrose, 2mM MgSO₄, 2mM sodium pyruvate, 4mM Mg-ATP, 0.4mM Tris GTP, 7mM phosphocreatine

disodium, 25 μ M Alexa 594, pH 7.3, osmolarity 290mOsm). The baseline recordings were performed 10 minutes after the cortical layer 5 pyramidal neurons were patched with an amplifier (Multiclamp 700B, Molecular devices) and digitized at 20kHz (Digidata 1550B, Molecular devices). Neurons with access resistance larger than 30M Ω or leak current larger than 300pA (at -75mV voltage clamp) were discarded. Then, another borosilicate puff pipette (10 to 15 μ m in diameter) filled with n-PBDF or PEDOT-PSS was moved to 20 to 30 μ m from the cell body, and a gentle positive pressure (0.2 to 0.4 psi) was applied briefly (~ 1 minute) to apply the polymer. The attachment of polymer on the neuron membrane was visually confirmed with a 40x water immersion objective (0.8 NA, Nikon) and an infrared (IR) camera (IR-2000, Dage-MTI) illuminated with a halogen lamp band-pass filtered at 600nm to 850nm. The electrophysiological recordings with puffed polymer were conducted in darkness to minimize thermo- or photoacoustic-related effects.

Two-photon optical stimulation: A two-photon microscope (Bruker Nano) with a femtosecond pulse laser (Insight X3, Spectra Physics) and two independent paths for imaging and photo-stimulation was used for optical modulation of neural activities through n-PBDF. Laser beam intensities were controlled with electro-optical modulators (model 350-50; Conoptics). Once the neurons were patched and filled with fluorescent dye, n-PBDF was puffed near the main apical trunk under a visual guide with overlaid fluorescent and Dodt Gradient Contrast (DGC) IR images under 810nm laser illumination. Photo-stimulation was performed with the fixed wavelength (1040nm) path of the InsightX3 laser with a set of galvanometric mirrors. Photo-stimulation with 720nm wavelength was performed with a separate femtosecond pulse laser (MaiTai, Spectra Physics) coupled to the galvanometric mirror path. The laser was scanned using a spiral pattern (20 μ m diameter, 5 to 20 revolutions) on n-PBDF adjacent to the dendrite. Hard shutters were used in addition to the electro-optical modulator to prevent prolonged laser exposure. The resting membrane potential (RMP) and dendritic morphology of the patched neurons were monitored throughout the optical modulation. Neurons showing signs of photodamage (dendritic blebbing, elevated RMP, elevated intracellular Ca²⁺ concentration, etc.) were discarded. For Ca²⁺ imaging, linescan (10 μ s dwell time per pixel, ~1000Hz) was performed under 810nm laser illumination across the dendritic branch adjacent to n-PBDF. The Ca²⁺ dynamics were filtered using a Savitzky-Golay finite impulse response filter and expressed as dG/R (calculated as (Green

- $\text{Green}_{\text{baseline}}/\text{Red}_{\text{baseline}}$, green denoting the functional fluorescence and red the structural fluorescence).

Characterization of neural passive and active properties: To measure the membrane capacitance and membrane resistance, a series of square currents with -50pA to 50pA amplitude and 500ms duration were injected during the whole-cell current clamp. The membrane resistance was computed as the slope of the I-V curve measured at a steady state. The membrane time constant was then computed by fitting the initial capacitive charge to the following equation, in which a and τ are fitting parameters:

$$V_m = a * (1 - \exp\left(-\frac{t}{\tau}\right))$$

The membrane capacitance was estimated based on the membrane resistance and time constant:

$$\tau = R_m C_m$$

To measure the Na^+ and K^+ current, step voltage changes from -75mV to 0mV or 30mV (1s duration) were sent under a voltage clamp. Na^+ current was estimated as the initial transient inward current (measured at the first 25ms after the step change of voltage), and K^+ current was estimated as the steady state outward current (the last 10 to 25ms at the end of the 1s step voltage). To measure the steady state gating properties of Na^+ and K^+ channels, voltage steps starting at -75mV going to various voltages (-90mV to 50mV, 300ms for Na^+ channels, and 1s for K^+ channels) were applied under a voltage clamp. Na^+ currents were measured by subtracting between the control and bath application of 1 μ M tetrodotoxin (TTX). K^+ currents were measured under bath application of 1 μ M TTX and a cocktail blocker (100 μ M CdCl_2 , 100 μ M NiCl_2 , and 5 μ M nifedipine) that blocks the voltage-gated Ca^{2+} channels. The leak conductance was estimated from hyperpolarization voltage (-90mV to -70mV) and subtracted from the K^+ conductance to get the putative voltage-gated K^+ channel conductance. The reversal potentials were $E_{\text{Na}} = 53.9\text{mV}$, $E_{\text{K}} = -85\text{mV}$ and $E_{\text{leak}} = -75\text{mV}$. The steady-state conductance as a function of voltage was fitted to a Boltzmann function (G , V_h , and k were fitting parameters):

$$g = \frac{I}{V_m - E}$$

$$g(V_m) = G_{\text{max}} * \frac{1}{1 + \exp\left(-\frac{V_m - V_h}{k}\right)}$$

To measure the recovery of Na⁺ channels, two voltage steps (from -75mV to 0mV, 1s duration) were applied with different time intervals (Δt). The maximum Na⁺ conductance measured at the second voltage step was fitted to an exponential function (a, b, and τ were fitting parameters):

$$g(\tau) = b - a * \exp\left(-\frac{\Delta t}{\tau}\right)$$

The codes for analysis of electrophysiological recordings are available on GitHub: <https://github.com/shulanx1/ConductivePolymer>.

Numerical simulation of neuronal optical modulation effects: A simplified three-compartment (soma-AIS, proximal apical dendrite, and distal apical dendrite) biophysical model of L5 PN was used to simulate the photo-stimulation-induced thermal and ionic modulation. The thermal modulation was simulated as shunting conductance, and the ionic effect was simulated as changes in the recovery time constant of dendritic Na⁺ channels. The differential equations are solved with the forward Euler method with an interval of 0.1 ms. The simulation was implemented with custom-written python code available on GitHub: https://github.com/shulanx1/simpl5pn_polymer.

Local temperature measurement with glass electrode: Local temperature change induced by NIR stimulation on n-PBDF/brain tissue was measured using glass pipette resistance (69). The pipette resistance was measured with 10mV pulses using a multiclamp 700B amplifier (Molecular devices). Pipette resistance as a function of temperature change was first calibrated with a heated ACSF bath and a thermocouple. The ACSF was first heated to around 50 °C and then gradually cooled down to calibrate the resistance-temperature curve. The following function was used to fit the resistance-temperature relationship (a was the fitting parameter, and T₀ and R₀ denote the baseline temperature and resistance, respectively) (**Fig. S76**).

$$\frac{1}{T_0} - \frac{1}{T} = a * \log\left(\frac{R_0}{R}\right)$$

Electrochemical impedance spectroscopy (EIS): The impedance changes induced by NIR stimulation on n-PBDF were measured using an impedance analyzer (EmStat 4S, PalmSens). The electrodes (reference electrode: Ag/AgCl, counter electrode: platinum and working electrode: ITO coated glass) were submerged in unpurified aqueous solution of n-PBDF (synthesized using Hb in ACSF), while NIR stimulation was performed with parked and expended (by the scan lens – tube

lens assembly on the 2p microscope) infinity-corrected NIR laser beam (1040nm). The impedance was measured from 0.1 Hz to 200 kHz.

In vivo silicon probe recording: Male and female C57BL/6J mice (12 to 24 weeks old) were used for *in vivo* measurement of neural spiking with n-PBDF injections. The animals were head-plated at least 24 hours before the experiment. On the day of the experiment, the animal was anesthetized with isoflurane, and a small craniotomy (<1mm) was made over the primary motor cortex (0.4mm AP, 1.5mm ML). A 64-channel electrode (Masmanidis Lab, UCLA) was inserted perpendicular to the surface of the pia using a micromanipulator (Sensapex 4-uMP, Finland). The microelectrode was positioned 1mm into the cortex, and a glass pipette (30µm tip diameter) containing n-PBDF was inserted at a 30-degree angle 300 µm below the brain surface. The silicon probe was allowed to settle for 20 minutes before recordings began. Signals were digitized at a bandwidth of 0.1 to 10 kHz and sampled at 20 kHz (Intan RHD, USA). Baseline neural activities were recorded for 30 to 45 minutes before n-PBDF was injected into the brain with a pressure injector, and the neural activities were recorded for another 30 to 45 minutes. After the recording session, we subjected electrical signals to processing, including sorting action potentials from individual neurons. This sorting was accomplished using Kilosort3 (<https://github.com/MouseLand/Kilosort>) and a meticulous manual review in Phy2 (<https://github.com/cortex-lab/phy>). Neurons were evaluated based on waveform characteristics, stability of firing rates observed throughout the recording session, and auto-correlograms. Spiking analysis was conducted on only neurons exhibiting sustained activity throughout the entire recording session. Neurons without stereotypical spiking waveforms, sustained activities throughout the recording session, or any reflection of the refractory period in the auto-correlograms are excluded from the analysis. Average neural activity for each animal for baseline and injected periods was smoothed by calculating the mean spiking activity across multiple trials per recording and convolving by a Gaussian filter with a full width at half-maximum (FWHM) width of 10 ms. The depth at which each spike occurred was determined based on the electrode that recorded the maximum waveform amplitude. For LFP analysis, data were down-sampled to 1000 Hz and bandpass filtered using a Butterworth filter from 1 to 200 Hz. Power spectral density plots were calculated using the Welch function with a moving window of 1000ms with 200 ms overlap.

In vivo polymerization in the mouse brain: Male and female C57BL/6J mice (12 to 24 weeks old) were used. The mice were anesthetized with isoflurane, and small craniotomies were

performed bilaterally over the primary motor cortex or primary somatosensory cortex. For in vivo polymerization, the mixture of BDF (10mM) and hemoglobin (0.1mol%) in dispensing buffer (ACSF (concentration in mM: 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 10 glucose, 2 MgCl₂, 2 CaCl₂), with 5 vol% DMSO and 1 wt% TPGS-750-M) was stirred at 37 °C for ~30 minutes to initiate the reaction, before being injected into the brain using a pulled glass pipette (500 nL, 25 nL per minute, 300 µm below the brain surface). After injection, the scalp was sutured, and the animals were kept in their home cage for incubation periods ranging from 2 to 14 days, before the animals were euthanized and the brains were collected for recordings or immunohistochemistry.

Cytokine assay: The subcutaneous and intracranial injections were performed as described earlier. The animals were euthanized one week after injection, and the brain/skin samples from the injection site were collected. Brains and skin samples were homogenized on ice in Dulbecco's phosphate-buffered saline (DPBS) containing Halt™ Protease Inhibitor Cocktail (100×; Thermo Scientific, Cat. No. 87786) using a dounce homogenizer. Homogenates were centrifuged at 4 °C, and clarified supernatants were collected for analysis. Cytokines were quantified with the LEGENDplex™ Mouse Inflammation Panel (13-plex) (BioLegend, Cat. No. 740446) according to the manufacturer's instructions. Samples were acquired on a 4-laser Attune™ NxT Flow Cytometer (Thermo Fisher Scientific) and analyzed using the LEGENDplex™ Data Analysis Software (Qognit platform).

Immunohistochemistry: The mice were anesthetized with isoflurane before trans-cardiac perfusion with ACSF, decapitation, and extraction of the brains. The brains are post-fixed overnight in 4% PFA solution, and cryoprotected in 30% (w/v) sucrose in water at 4°C. Coronal sections (40 µm thick) were prepared using a frozen sliding microtome (Thermo Scientific Microm HM430). Sections were stored at - 20°C in cryoprotectant solution (30% w/v sucrose and 30% v/v ethylene glycol in 0.1 M phosphate buffer, pH 7.4) until IHC analysis. Free-floating sections were washed 3 times for 10 min each in PBS to remove residual cryoprotectant solution and then incubated in PBS containing Triton X-100 (1% v/v) for 60 min. The sections were blocked in a solution of 10% (v/v) normal donkey serum in PBS supplemented with Triton X-100 (0.3% v/v) (PBST) for 90 min and then incubated with primary antibody solution prepared in PBST containing 1% (v/v) normal donkey serum overnight at 4 °C. After washing in PBS (3 x 10 min), the sections were incubated with Alexa fluor-conjugated secondary antibodies (Jackson Immuno-Research

Laboratories, West Grove, PA; 1:500 dilution) at 22°C for 90 min. After a final round of three 10-min washes in PBS, the tissues were mounted on slides, allowed to dry overnight, and sealed with coverslips using DPX mounting media (Electron Microscopy Sciences #13512). The primary antibodies used in these experiments included antibodies specific to GFAP (Cell Signaling Technologies #3670; Danvers, MA; 1:500), NeuN (GeneTex GTX 00837; Irvine, CA; 1:1000), and Ki-67 (MA5-14520, Invitrogen, 1:500). The stained and mounted sections were imaged with a confocal microscope (A1Rsi, Nikon), and the puncta analysis was performed using QuPath (<https://qupath.github.io>) (70).

Motor learning task under photo-stimulation of n-PBDF: Male and female C57BL/6J mice (12 to 24 weeks old) were used for the lever-pull behavioral task. The animals were head-plated, and a 3mm diameter cranial window was performed over the primary motor cortex with n-PBDF injection (250nL per injection site, four injection sites across the window). The animals were allowed to recover for at least 24 hours before water restriction and training. Water restriction was initiated 1 to 2 days prior to training, during which mice were extensively habituated to the experimental and behavioral setup. Daily water intake was monitored at 1.2 mL per day. Throughout this period, mouse weight was closely monitored, ensuring no more than a 20% loss from their initial body weight.

After 20 days of n-PBDF injection, Mice underwent daily training sessions for 5-7 days, performing a lever-pull task. A Hall-sensor gimbal (NanoGimbal) tracked the lever's position, with its output sampled at 100 Hz by a custom Arduino-based system. This system interfaced with a custom Python script to monitor lever trajectories and task performance. During training, mice used their right paw to grasp the lever, while their left paw rested on a stationary block. A successful lever pull to a set threshold resulted in a reward of approximately 10 µl of water. This was followed by a variable inter-trial interval (ITI) lasting 3 seconds. Lever pulls made during the ITI resulted in a reset of the ITI. Each training session consisted of 100 to 200 trials.

Photo-stimulation was performed once the mice exhibited a precise lever-pulling movement above the set threshold (typically requiring 5 to 7 training sessions). The photo-stimulation was performed using a two-photon microscope (MOM, Sutter Instrument) and a 920nm femtosecond pulse laser (Insight X3, Spectra-Physics). The injection site with n-PBDF was located using an IR-sensitive camera. Baseline behavioral performance was recorded for 10 to 20 minutes prior to the

stimulation. During photo-stimulation, the stimulation beam (30mW, 920nm) was continuously scanned (30Hz, 512*512 pixels) over a 500 μ m*500 μ m FOV, 300 μ m below the brain surface, with a resonance galvo scanner.

Blood vessel imaging: The animals were head-plated, and a 3mm cranial window was performed 24h prior to the first imaging session. For the vasculature imaging, 2.5% (w/v) fluorescein (100 μ L) was injected into the tail vein. The imaging (500 μ m $\times$ 500 μ m $\times$ 400 μ m, 512 $\times$ 512 pixel, 5 μ m step size in depth) was performed immediately after the fluorescein injection using a two-photon microscope (MOM, Sutter Instrument) and a 920nm femtosecond pulse laser (Insight X3, Spectra-Physics). After the first imaging session, the animals were anesthetized and intracranially injected with a mixture of BDF (10mM) and hemoglobin (0.1mol%) in dispensing buffer (ACSF, with 5 vol% DMSO and 1 wt% TPGS-750-M) after initiating the reaction for around 30 minutes. The imaging was performed again at 5h, 24h, 3 days, and 1 week after injection. The diameter, length, and volume of the blood vessels were extracted using VesselExpress (71). To remove false positives during skeletonization, vessels with a length below 150 μ m or a volume below 500 μ m³ were excluded. The blood-brain-barrier permeability was quantified as the intensity of background fluorescence over the intensity of blood vessels. The permeability was calculated for each depth, and the median values across all depths were reported.

Two-photon glutamate uncaging: The two-photon glutamate uncaging on brain slices was performed similarly to previous reports (49). 2.5mM MNI-caged-L-glutamate (Tocris) was suspended in the ACSF bath for uncaging. The glutamate uncaging was performed using a laser-scanning microscope (Bruker Nano) with a tunable femtosecond pulsed laser (720nm, 1ms pulse, 20mW at the sample plane) (Mai-Tai, Spectra Physics). The amplitude and half-width of excitatory postsynaptic potentials (EPSPs) evoked by uncaging on basal dendritic synapses of L5 pyramidal neurons were extracted with custom-written Matlab codes. To assay the cooperation of synapses, the neighboring dendritic spines (8 spines within a 15 μ m basal dendritic segment) were semi-simultaneously stimulated (interval 0.26ms), and the EPSP amplitude as a function of the number of coactivated synapses was fitted to a sigmoidal curve.

Mice open-field behavioral assay: Open-field and rearing analysis were done similarly to previous reports (72). Mice were tested on comprehensive behavior for 4 days. The order of tests was randomized, and all tests were conducted by an experimenter blinded to the treatment group.

All tests were conducted between 08:00 and 4:00 in the lights-on cycle. Mice were habituated to the testing room 1 h before tests, and the apparatus was cleaned with 70% ethanol in between animals to minimize odor cues.

To assess general activity, locomotion, and anxiety, mice were tested in the open field. Mice were individually placed in the center and allowed to freely explore for 10 min while the trial was videotaped. Subsequent video scoring was completed by an observer blind to treatment groups using Bonsai and custom-coded MATLAB scripts to analyze exploration index and rearing time. The exploration index was calculated as the time each mouse spends in a defined area within the arena (a value of -1 denotes very high occupation in a single region, while 1 denotes brief occupation in a single region). Rearing time was calculated by the time the mouse spent above its situated height during active exploration.

Statistical analysis: Data analysis and statistical tests were performed using custom-written MATLAB (MathWorks, USA) and Python code. When two-way repeated measures ANOVA were performed (**Figs. 4F, 4J, 4O, 5C, 6E, S51**), the *p* values for interaction between the two factors (ie. the presence of n-PBDF and the amount of injected current in **Fig. 4F**) were reported, and the degrees of freedom were reported in the brackets. For the *in vivo* silicon probe recording (**Fig. 4Q**), nested factors (ie. different neurons recorded from the same animal) were specified in the statistical tests (73). Unless stated otherwise, the error bars represent mean $\pm$ standard deviation (SD), and the box plots represent median, 25th, and 75th percentiles. All figures with data collected from biological samples reflected biological replicates.

Zebrafish blood injection assay (PTU-treated): The zebrafish husbandry and experiment were conducted in accordance with internationally accepted standards. The Animal Care and Use Protocol was approved by the Purdue Animal Care and Use Committee (PACUC), adhering to the Guidelines for using Zebrafish in the NIH Intramural Research Program (protocol number: 1401001018). The AB wild-type strain was used in this study. The zebrafish blood injection in this study is modified from the zebrafish vasculature infection assay as described previously (74). The larvae were treated with 200 μ M PTU (1-phenyl 2-thiourea) in E3 medium starting at 24 hours post-fertilization to inhibit pigmentation on the fish body. Briefly, 20 of 3 days post fertilization larvae in each group were injected with 1nL of the desired concentration of BDF monomer solution into the ventral vein while the sterile PBS was injected as control. Then, the fish larvae were

incubated at 34 °C for 24 hours before imaging. After 24 hours post-injection, the zebrafish were imaged by a Zeiss ZV16 microscope with a bright field and pooled by the group for downstream analysis. Representative experiments of three independent repeats were shown.

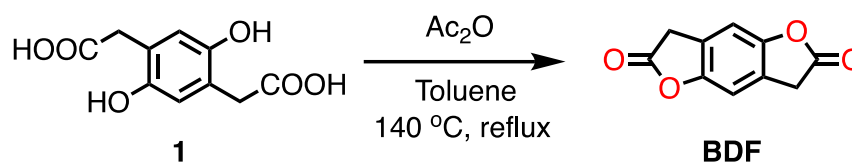
Zebrafish blood injection assay: The zebrafish husbandry and experiment was conducted in accordance with internationally accepted standards. The Animal Care and Use Protocol was approved by the Purdue Animal Care and Use Committee (PACUC), adhering to the Guidelines for using Zebrafish in the NIH Intramural Research Program (protocol number: 1401001018). The AB wild-type strain was used in this study. The zebrafish blood injection in this study is modified from the zebrafish vasculature infection assay described earlier. Briefly, 20 of 3 days post fertilization larvae in each group were injected with 1nL of the desired concentration of BDF monomer solution into the ventral vein while the sterile PBS was injected as control. Then, the fish larvae were incubated at 34 °C for 24 hours before imaging. After 24 hours post-injection, the zebrafish were imaged by a Zeiss ZV16 microscope with a bright field and pooled by the group for downstream analysis. Representative experiments of three independent repeats were shown.

Zebrafish c-turn behavioral assay: The c-turn assay was performed 3 to 5 days post-injection of BDF monomer. The larvae were transferred into a petri dish containing E3 media. To initiate the c-turning behavior, a cut hair loop was used to gently touch the head or back of the fish. The turning behavior was captured using a high-speed camera (photonfocus, DR1-D1312IE-200-G2) at 750 fps from a bottom view of the petri dish. The head, back, and tail of the larvae were automatically labeled on each frame using DeeplabCut with a manually labeled training dataset (around 200 frames extracted using k-means clustering). The labels were then manually inspected for accuracy. The head direction, tail direction, and body curvature were computed using the coordinates of the labels with custom-written Matlab codes. The turn time was calculated as the time taken from the beginning of the turn (turning larger than 1 degree between consecutive frames) to a more than 140 degrees change in head direction.

Zebrafish imaging and length quantification: Zebrafish larvae were measured at 3, 5, and 7 days post-fertilization (1, 3, and 5 days post-injection of BDF monomer). The larvae were anesthetized by being placed into a petri dish with E3 media containing 0.02% tricaine. Images were taken with an AXIO Zoom V16 microscope (Zeiss). Length was determined by measuring

from the head of the larvae to the end of the notochord in millimeters using ImageJ software. A total of 158 fish were measured for the experiment.

Synthesis of BDF monomer: BDF was synthesized via a previously reported method with slight modification (75).



To a suspension of compound **1** (1.0 g, 4.4 mmol) in anhydrous toluene (15 mL), Ac₂O (15 mL) was added. The mixture was stirred at 140 °C for 2.5 h, and then the reaction was cooled to room temperature slowly overnight. The recrystallized product was filtered under a vacuum and washed with cold toluene at least three times. The product was kept under high vacuum at room temperature to obtain BDF as a white crystal (0.65 g, 78%). ¹H NMR (DMSO-D₆, 400 MHz, ppm): δ 7.24 (s, 2H), 3.95 (s, 4H).

Synthesis of n-PBDF polymer with copper acetate: The BDF monomer was dissolved in a stock solution of 5% DMSO in PBS buffer with 1% w/v TPGS-750-M surfactant for 1 hour. After 1 hour, Cu(OAc)₂ was added to the solution mixture. The mixture was heated to 37 °C and stirred for 6 hours in the air. After the solution was cooled to RT, the mixture was dialyzed against 20% DMSO in DI water using a dialysis bag with a cut-off molecular weight of 10 kDa (ThermoScientific SnakeSkin Dialysis Tubing, USA) to remove low molecular weight fractions, copper catalyst, and additives.

Synthesis of n-PBDF polymer with Hemin: The BDF monomer was dissolved in a stock solution of 5% DMSO in PBS buffer with 1% w/v TPGS-750-M surfactant for 1 hour (25mM BDF concentration). After 1 hour, Hemin was added to the solution mixture. The mixture was heated to 37 °C and stirred for 6 hours in the air. After the solution was cooled to RT, the mixture was dialyzed against 20% DMSO in DI water using a dialysis bag with a cut-off molecular weight of 10 kDa (ThermoScientific SnakeSkin Dialysis Tubing, USA) to remove low molecular weight fractions, hemin, and additives.

Synthesis of n-PBDF polymer with Hemoproteins: The BDF monomer was dissolved in a stock solution of 5% DMSO in PBS buffer (or RPMI 1640 media with 10% FBS and 1% Penicillin-

streptomycin) with 1% w/v TPGS-750-M surfactant for 1 hour. After that, Hemoprotein was added to the solution mixture. The mixture was heated to 37 °C and stirred for 6 hours in the air. After the solution was cooled to RT, the mixture was dialyzed against 20% DMSO in DI water using a dialysis bag with a cut-off molecular weight of 10 kDa (ThermoScientific SnakeSkin Dialysis Tubing, USA) to remove low molecular weight fractions and additives for certain characterizations; otherwise, it was used further without dialysis.

Synthesis of n-PBDF polymer with RBCs/Whole Blood: The BDF monomer was dissolved in a stock solution of 5% DMSO in PBS buffer (or RBC buffer or RPMI 1640 media with 10% FBS and 1% Penicillin-streptomycin) with 1% w/v TPGS-750-M surfactant for 1 hour. After that, RBCs (suspended in RBC buffer)/Whole Blood were added to the solution mixture to a total volume of 4mL. The mixture was heated to 37 °C and stirred for 6 hours in the air. After the solution was cooled to RT, characterization was done without any further purification.

Synthesis of n-PBDF polymer using Whole Blood without stirring: The BDF monomer was dissolved in a stock solution of 5% DMSO in RPMI 1640 media with 10% FBS, 1% Penicillin-streptomycin, and 1% w/v TPGS-750-M surfactant for 1 hour. After that, whole blood was added to the solution mixture to a total volume of 4mL. The mixture was kept inside an incubator at 37 °C with slow rocking for 8 hours. After the solution was cooled to RT, characterization was performed without any further purification.

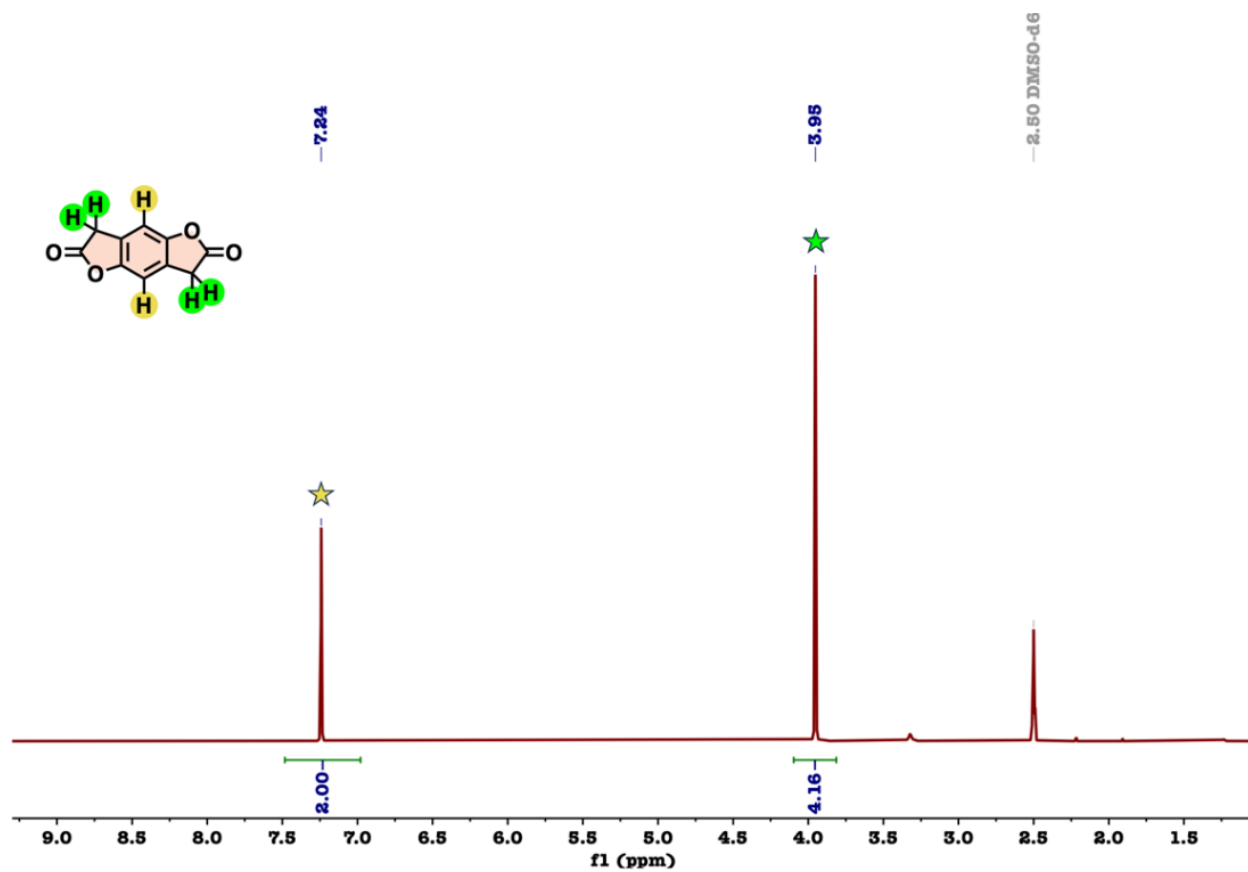


Figure S1. ^1H NMR of **BDF** monomer in $\text{DMSO-}D_6$.

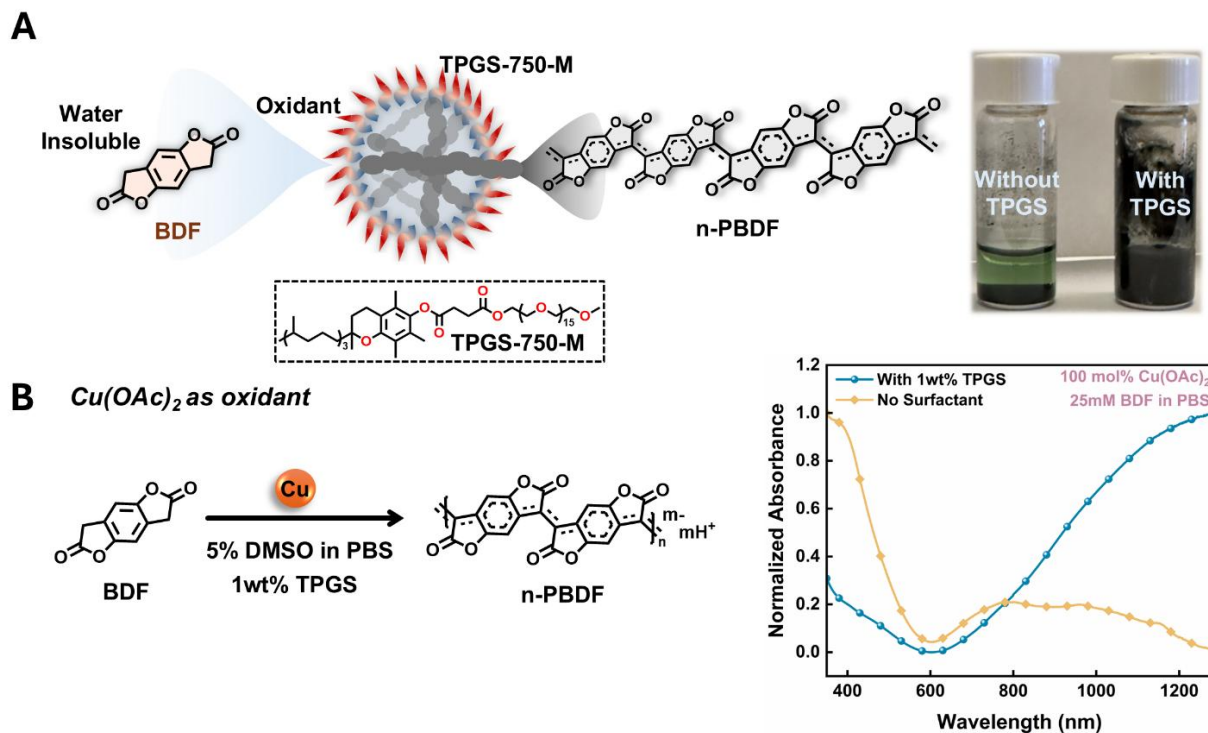


Figure S2. Synthesis of n-PBDF in aqueous media (A) Schematic representation of the biocompatible synthesis of n-PBDF in aqueous media at 37 °C using a surfactant, and (B) Copper-mediated polymerization in the presence/absence of TPGS-750-M as a surfactant.

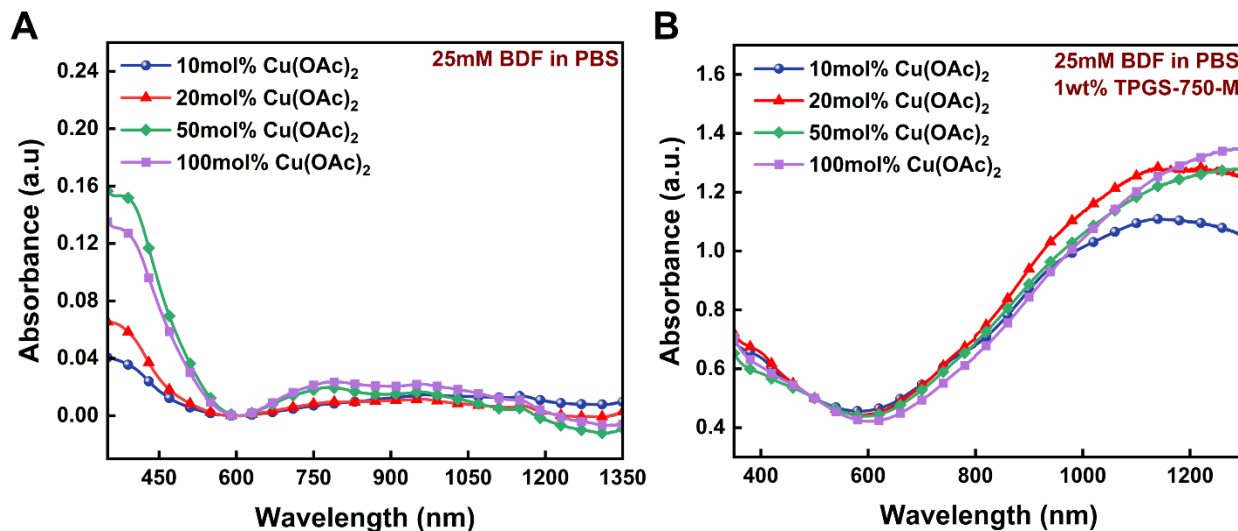


Figure S3. UV-Vis-NIR spectrum of n-PBDF polymer using copper acetate (A) without surfactant and (B) with 1wt% TPGS-750-M surfactant.

Using 1wt% TPGS-750-M surfactant, we can observe the formation of highly doped n-PBDF polymer, characterized by a strong polaron peak in the NIR region around $\sim 1150\text{nm}$. The intensity of the polaron peak increases as the amount of copper acetate increases.

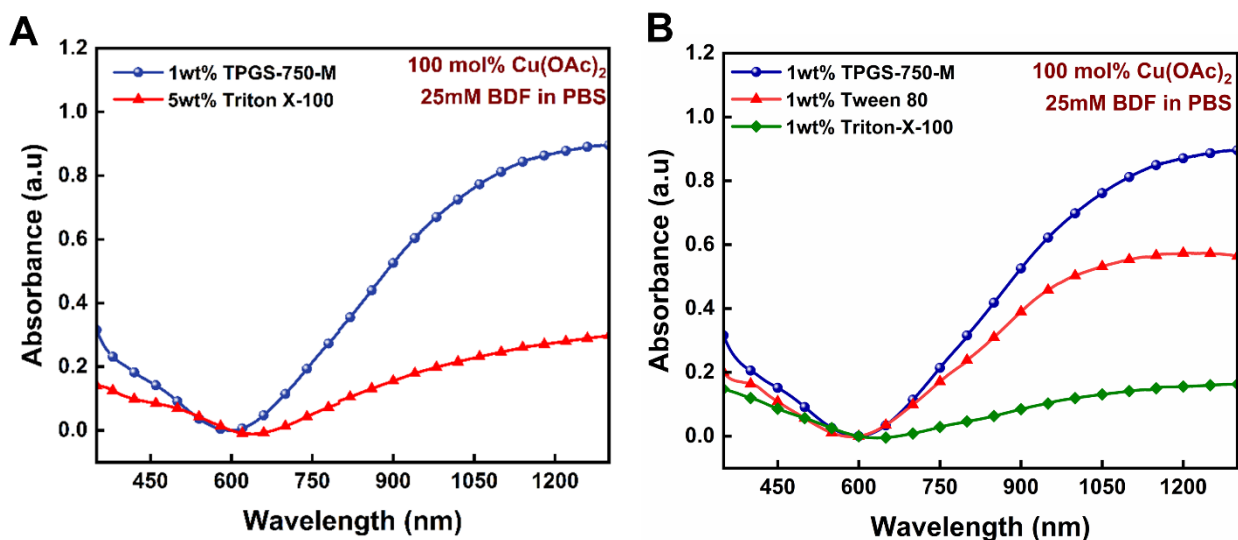


Figure S4. UV-Vis-NIR spectrum of n-PBDF polymer with different surfactants using copper acetate as catalyst. (A) TPGS-750-M vs Triton X-100, and (B) in 1wt% surfactants concentrations.

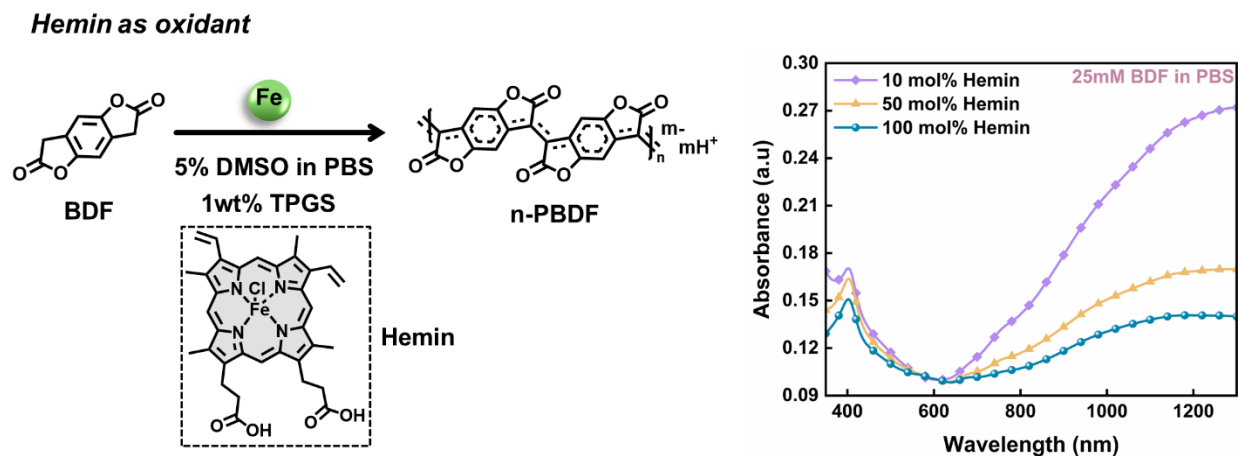


Figure S5. Hemin promoted polymerization and the de-doping phenomenon.

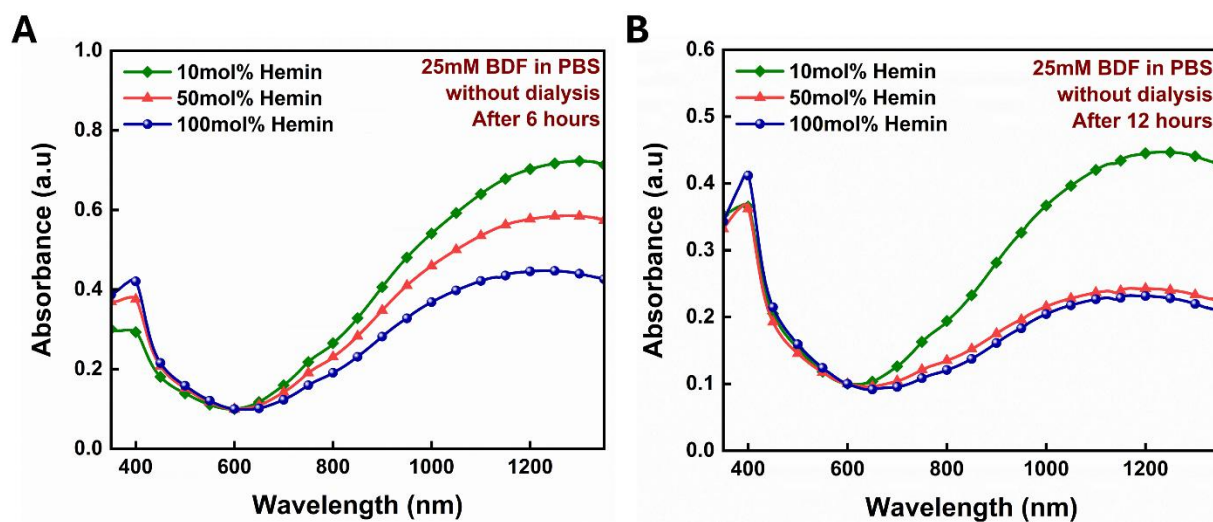


Figure S6. UV-Vis-NIR spectrum of n-PBDF polymer with hemin as the catalyst and without dialysis. (A) After 6 hours, and (B) After 12 hours.

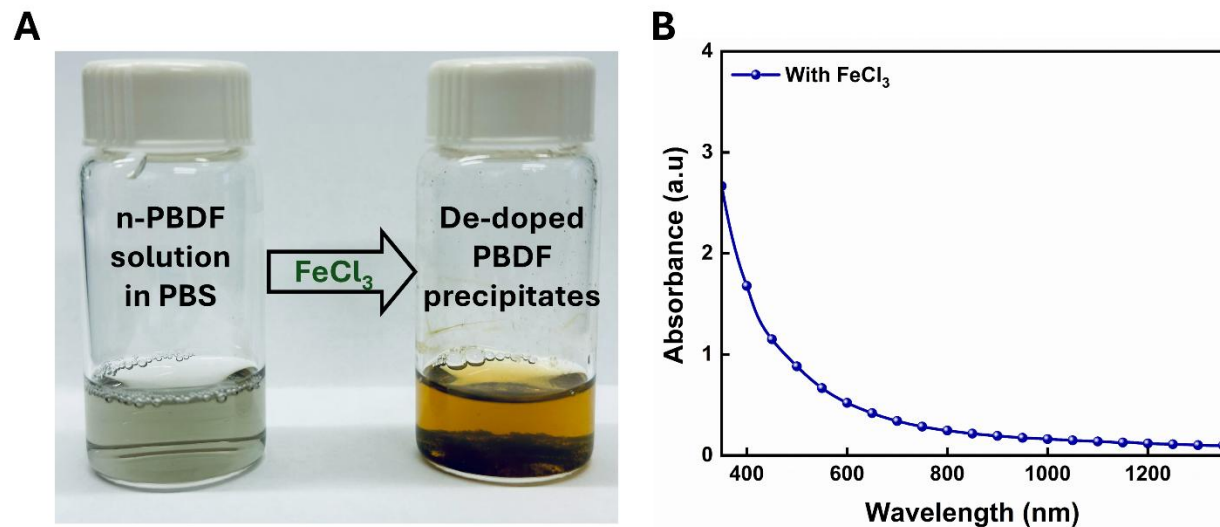


Figure S7. (A) Effect of FeCl_3 on the n-PBDF solution in PBS, (B) UV-Vis-NIR spectra after 12 hours of FeCl_3 addition.

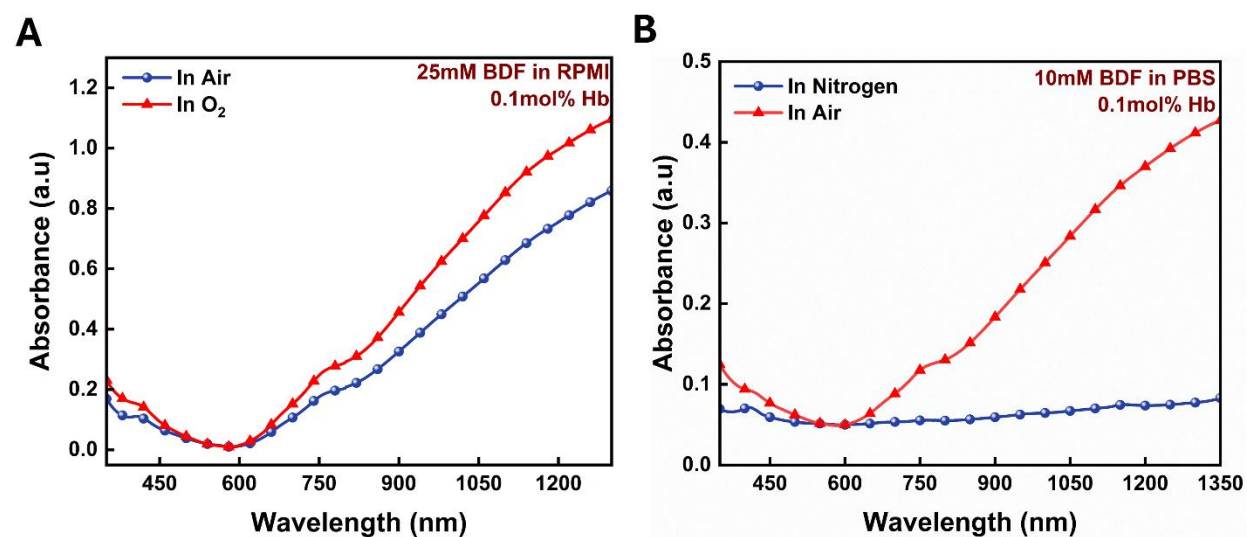


Figure S8. The UV-Vis-NIR spectrum of n-PBDF solution formed in (A) an air vs. oxygen-rich environment and (B) an air vs. inert-nitrogen environment.

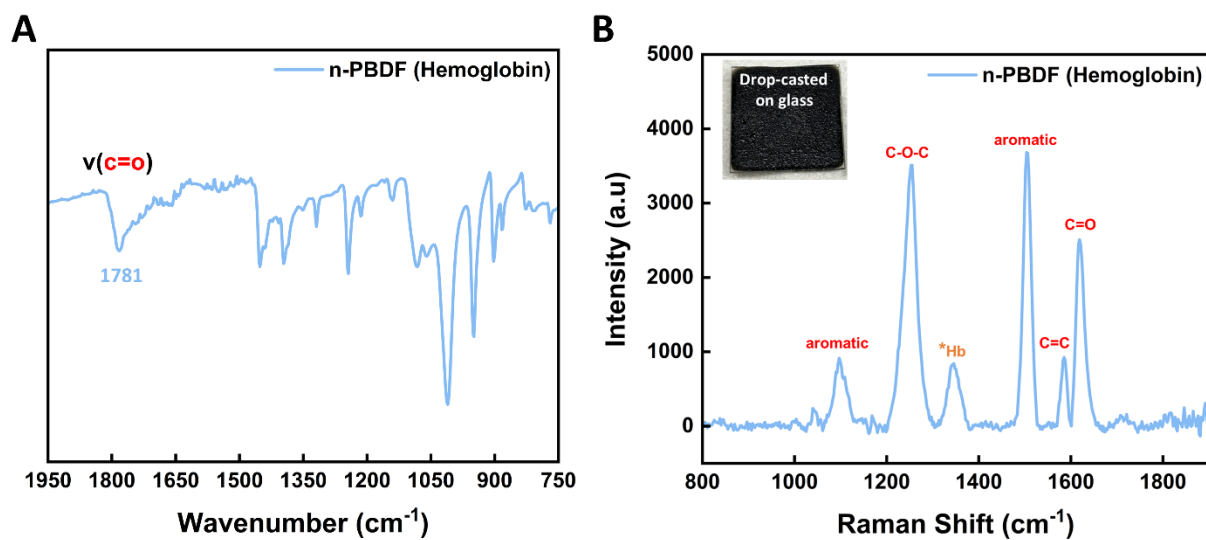


Figure S9. Structural characterization of n-PBDF synthesized by Hb without any purification (A) *In situ* FTIR spectrum of n-PBDF solution and (B) Raman spectrum of n-PBDF film (Inset: drop-casted n-PBDF film on glass substrate and vacuum dried at room temperature).

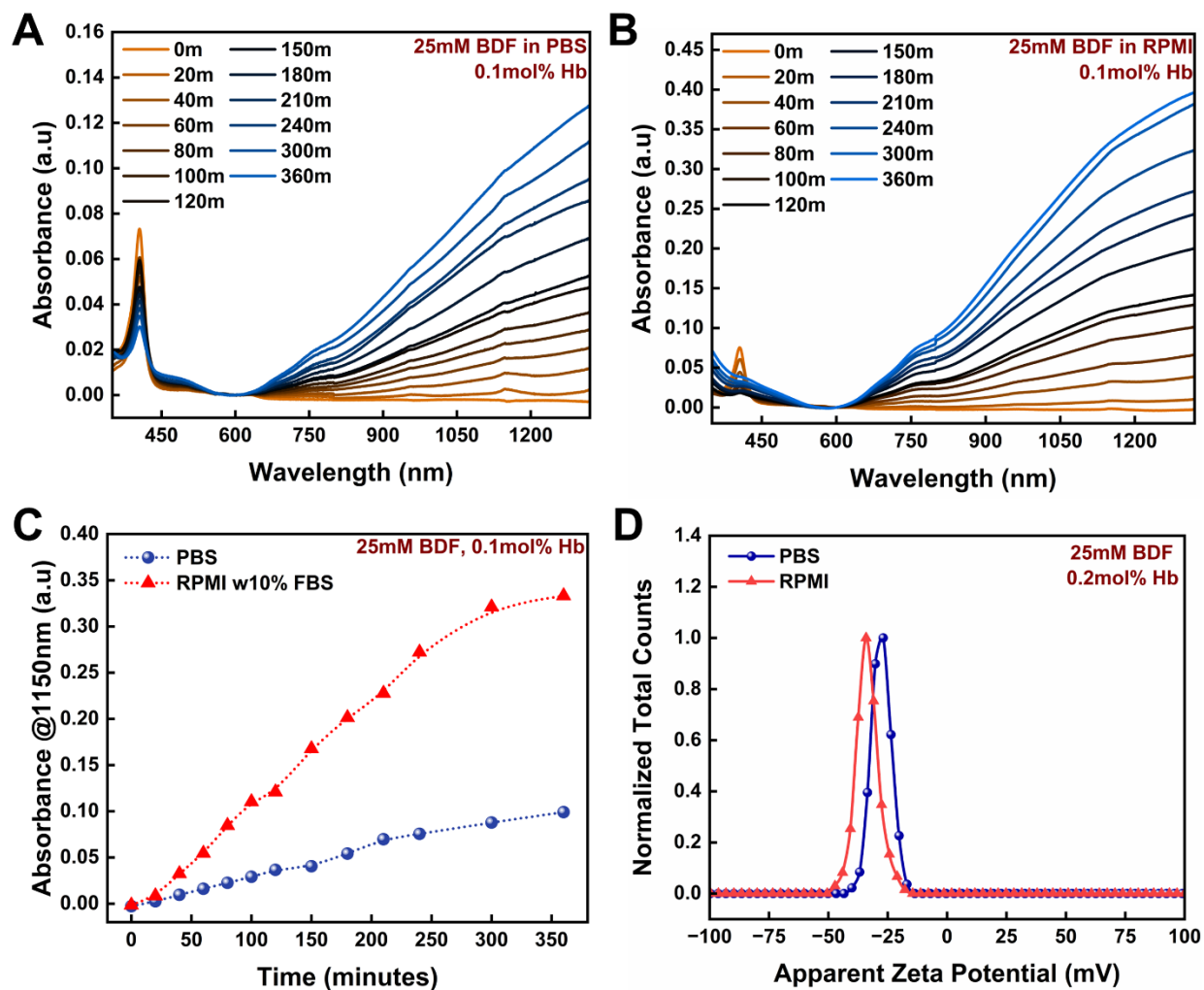


Figure S10. UV-Vis-NIR spectrum of n-PBDF polymer formation using 0.1mol% Hb as catalyst (A) In PBS Buffer, (B) In RPMI media, (C) comparison of n-PBDF polaron absorption with time in different solvents, and (D) comparison of ZP in different media with 0.2mol% Hb.

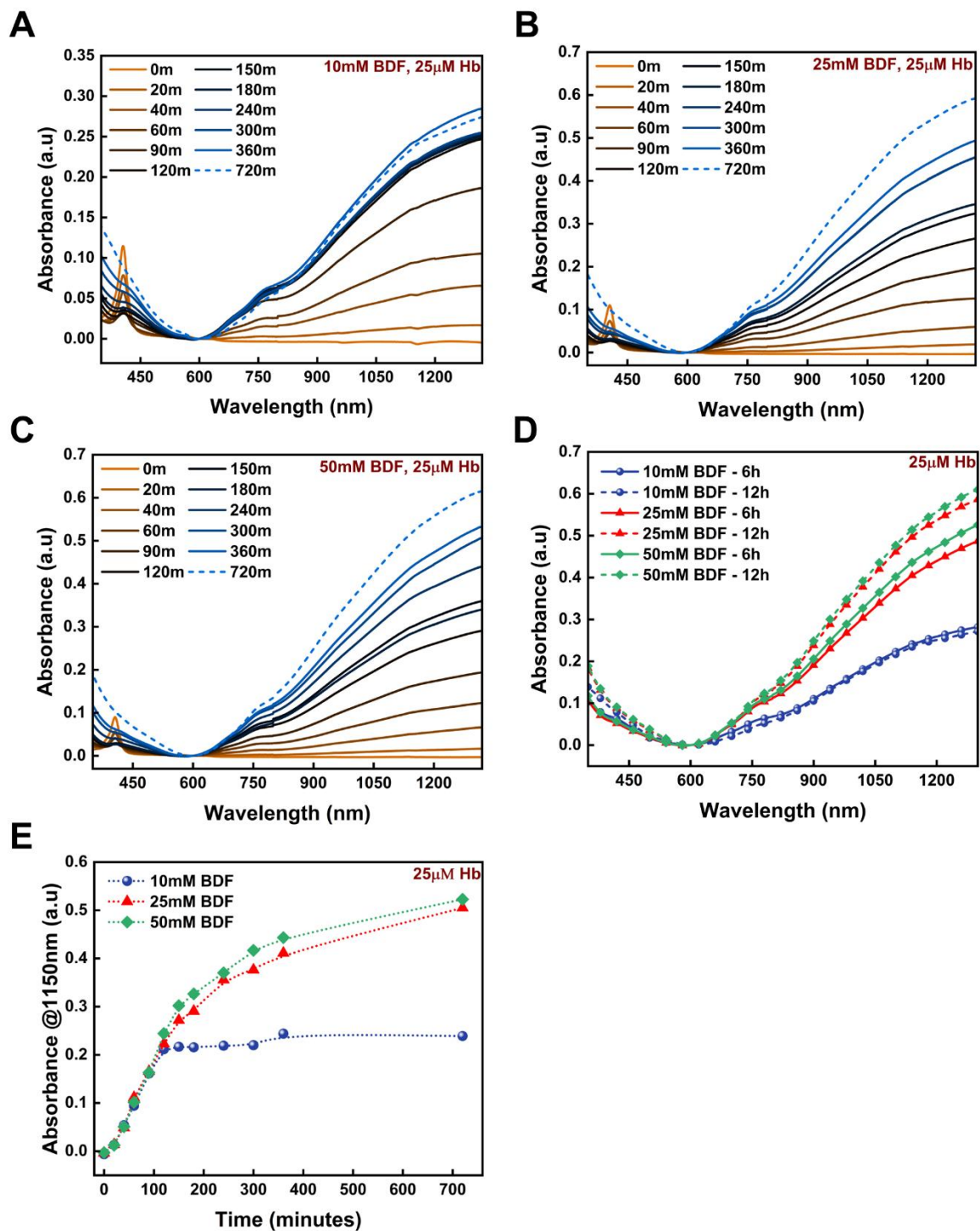


Figure S11. UV-Vis-NIR spectrum of n-PBDF polymer formation using 25 μ M Hb as a catalyst in RPMI (A) 10mM BDF, (B) 25mM BDF, (C) 50mM BDF, (D) comparison of n-PBDF polaron absorption at 6h and 12h, and (E) n-PBDF polaron absorption with time at varying BDF concentrations.

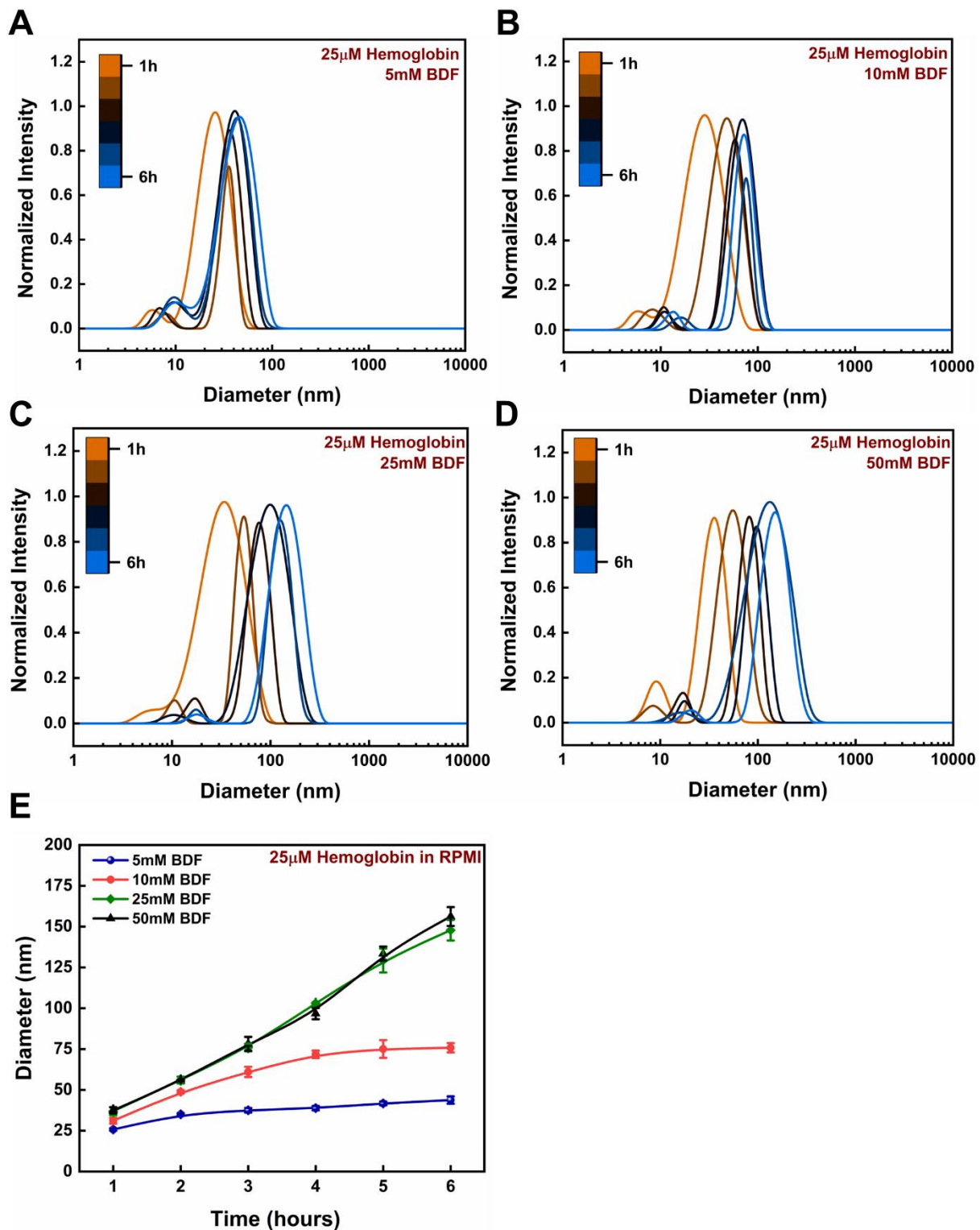


Figure S12. DLS spectrum of n-PBDF polymer formation using 25 μ M Hb as a catalyst in RPMI (A) 5mM BDF, (B) 10mM BDF, (C) 25mM BDF, (D) 50mM BDF, and (E) comparison of n-PBDF hydrodynamic diameter at varying BDF concentrations and time intervals.

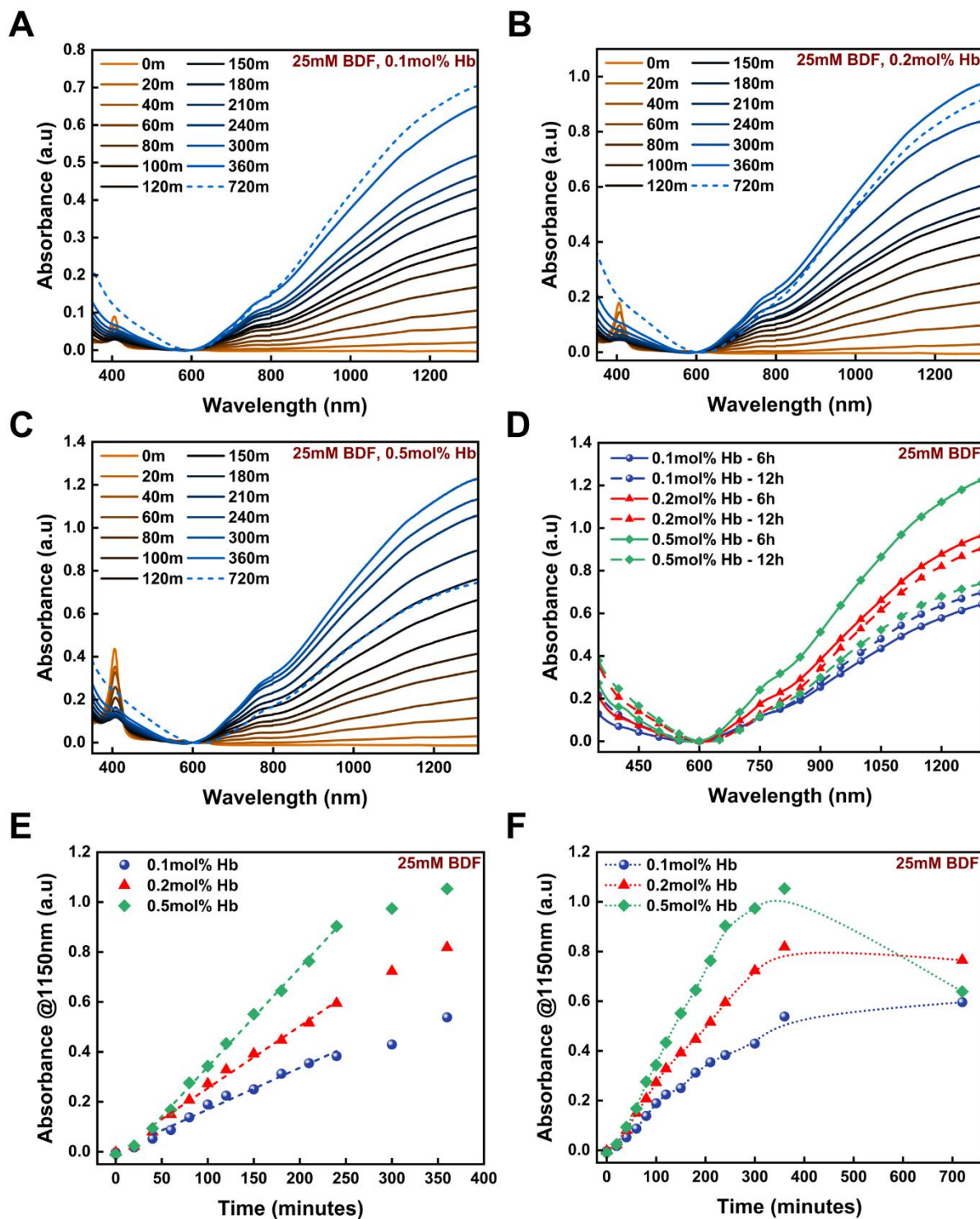


Figure S13. UV-Vis-NIR spectrum of n-PBDF polymer formation using Hb as a catalyst in RPMI with constant BDF concentration of 25mM (A) 0.1mol% Hb, (B) 0.2mol% Hb, (C) 0.5mol% Hb, (D) comparison of n-PBDF polaron absorption at 6h and 12h, and n-PBDF polaron absorption with time at varying Hb concentrations (E) In initial 6 hours (F) In 12 hours.

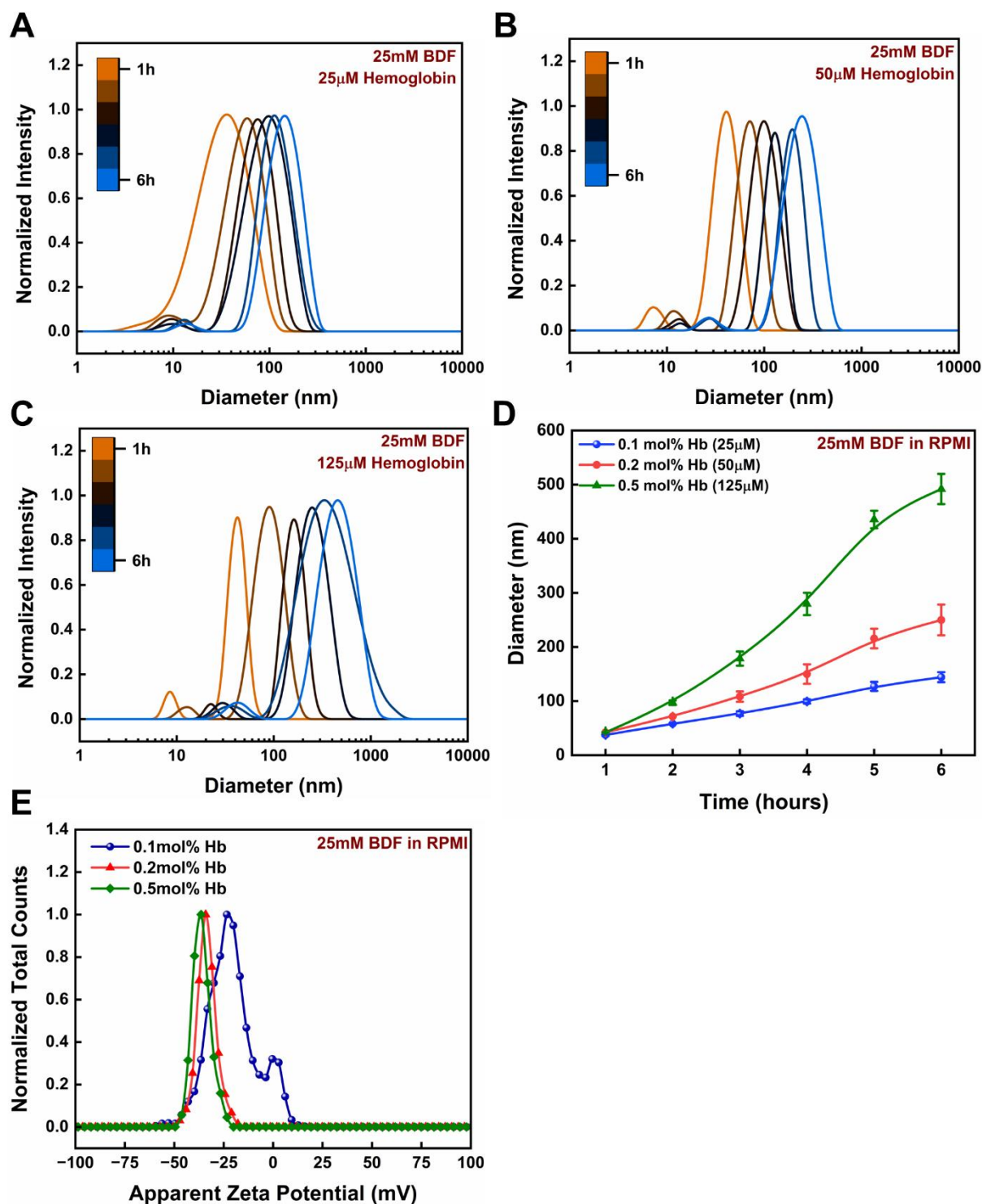


Figure S14. DLS spectrum of n-PBDF polymer formation using Hb as a catalyst in RPMI with constant BDF concentration of 25mM (A) 0.1mol% Hb, (B) 0.2mol% Hb, (C) 0.5mol% Hb, (D) n-PBDF hydrodynamic diameter with time at varying Hb concentrations, and (E) ZP spectrum at varying Hb concentrations.

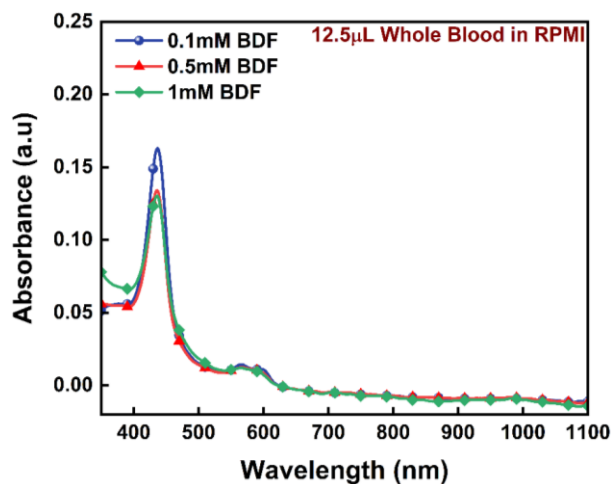


Figure S15. UV-Vis-NIR spectrum of n-PBDF using whole blood as a catalyst at very low BDF concentration.

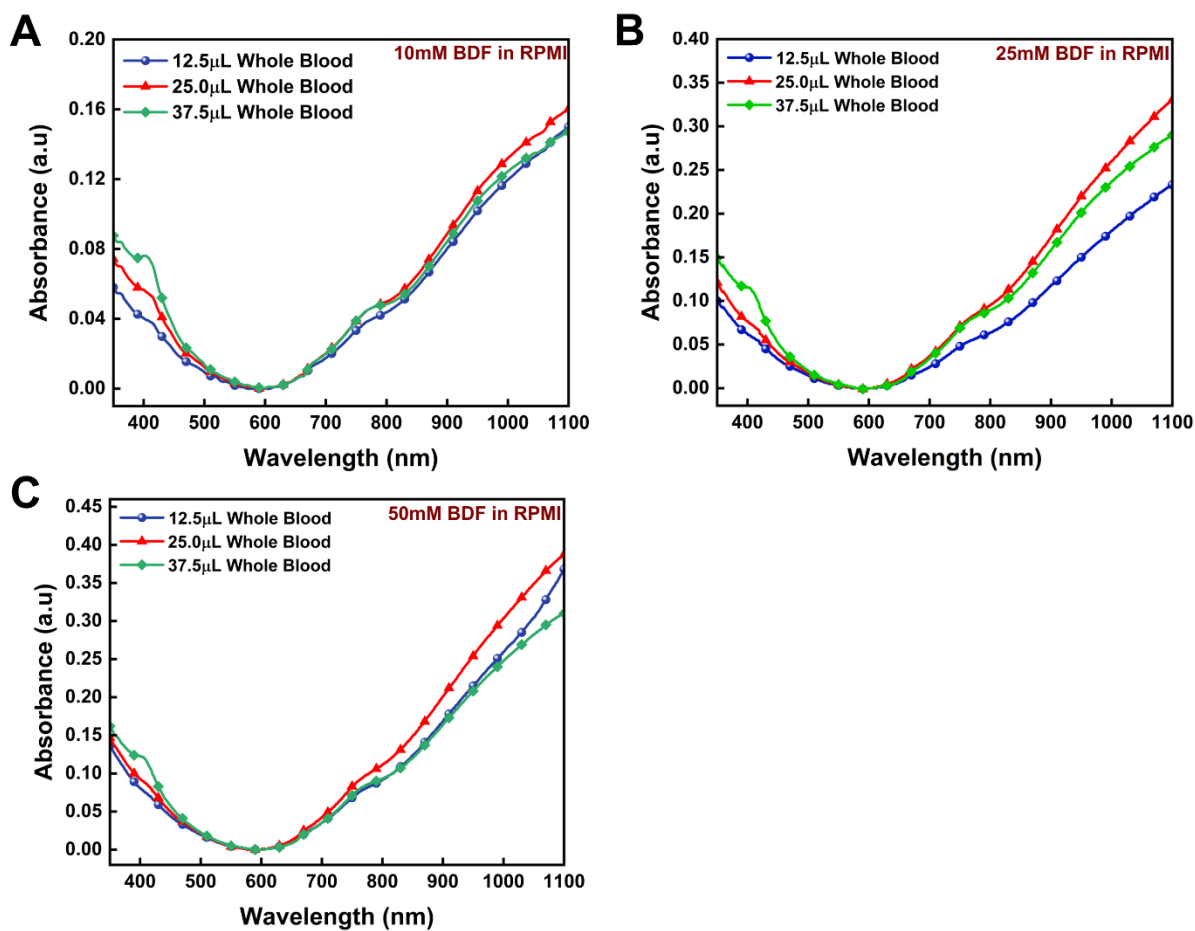


Figure S16. UV-Vis-NIR spectrum of n-PBDF at varying whole blood concentrations with (A) 10mM BDF, (B) 25mM BDF, and (C) 50mM BDF concentrations.

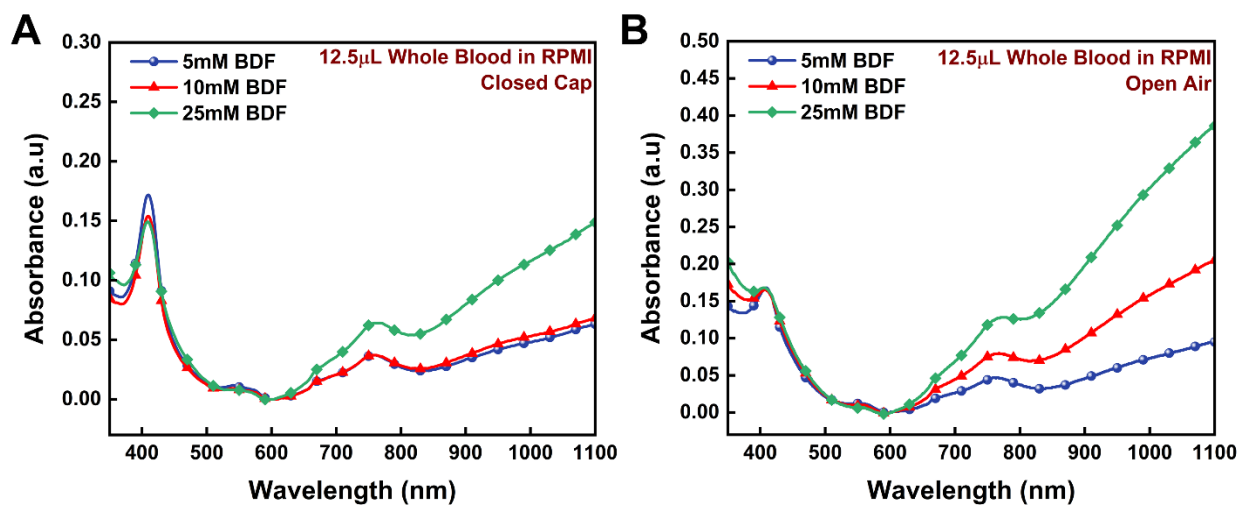


Figure S17. UV-Vis-NIR spectrum of n-PBDF at varying BDF concentrations in RPMI using whole blood as a catalyst inside an incubator without stirring (**A**) with a closed cap and (**B**) with an air balloon.

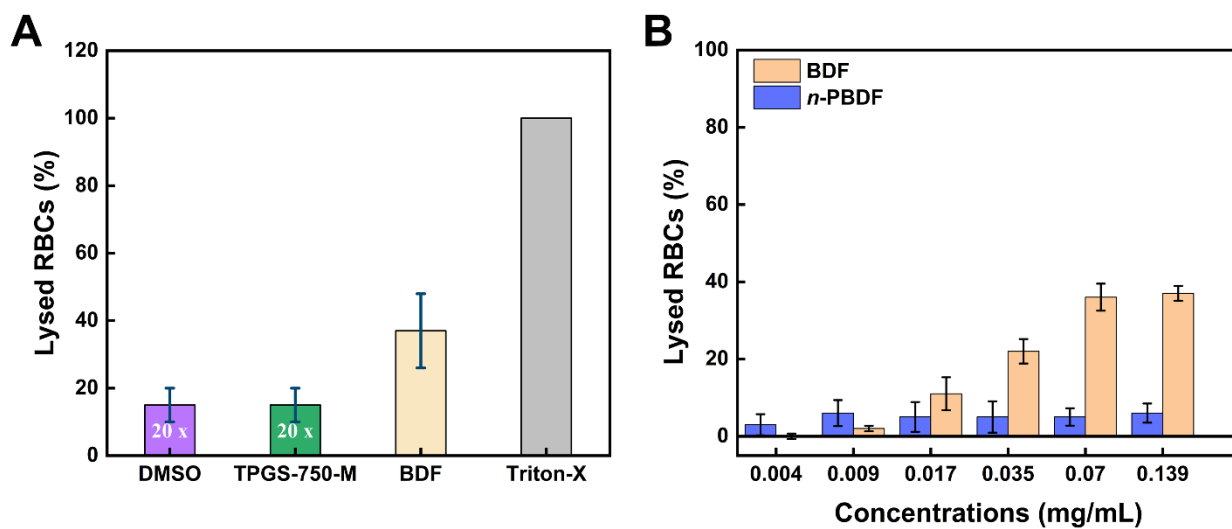


Figure S18. Hemolysis with (**A**) Reaction components (5% v/v DMSO, 1% w/w TPGS, 0.2 mg/mL BDF), and (**B**) BDF monomer and n-PBDF polymer

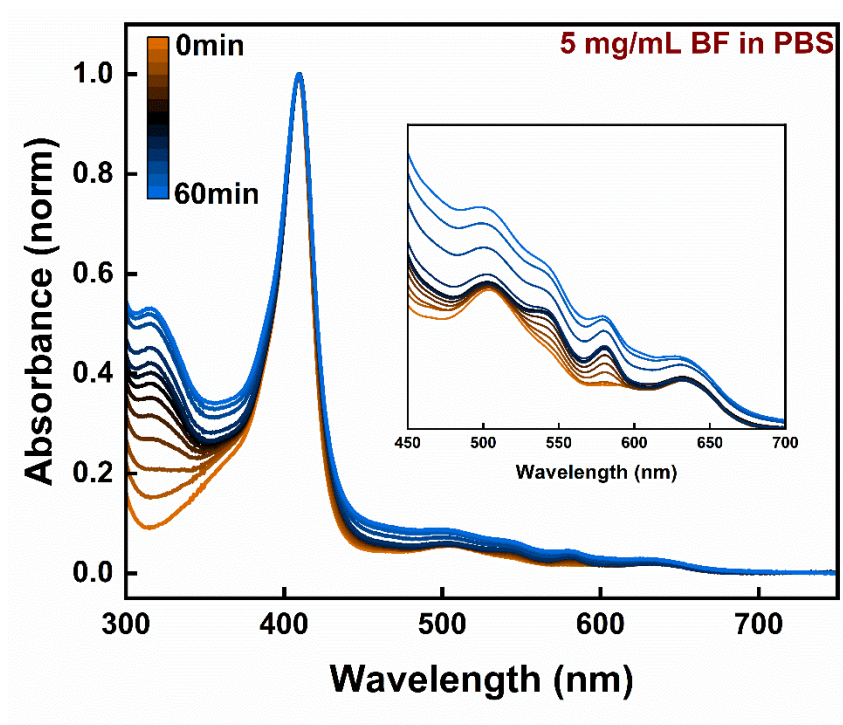


Figure S19. UV-Vis-NIR spectrum of Myoglobin (Mb) along with 2-Coumaranone (BF) in PBS and TPGS surfactant at different intervals at 37 °C. Inset: zoomed portion from 450nm – 700nm.

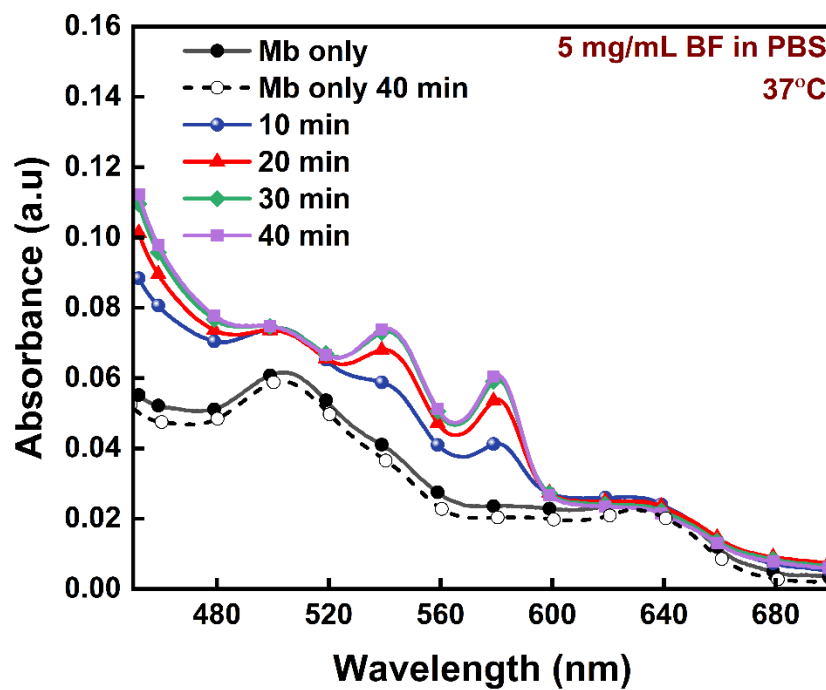


Figure S20. UV-Vis-NIR spectrum of Myoglobin (Mb) along with 2-Coumaranone (BF) in PBS without any surfactant at different intervals at 37 °C.

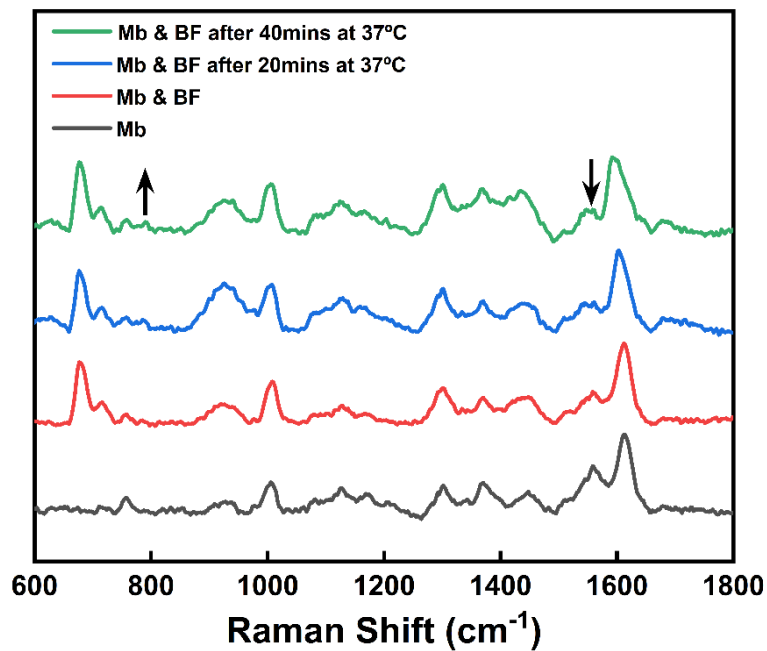


Figure S21. Raman spectrum of Myoglobin (Mb) and 2-Coumaranone (BF) in PBS with TPGS at different intervals, excitation with 532nm LASER.

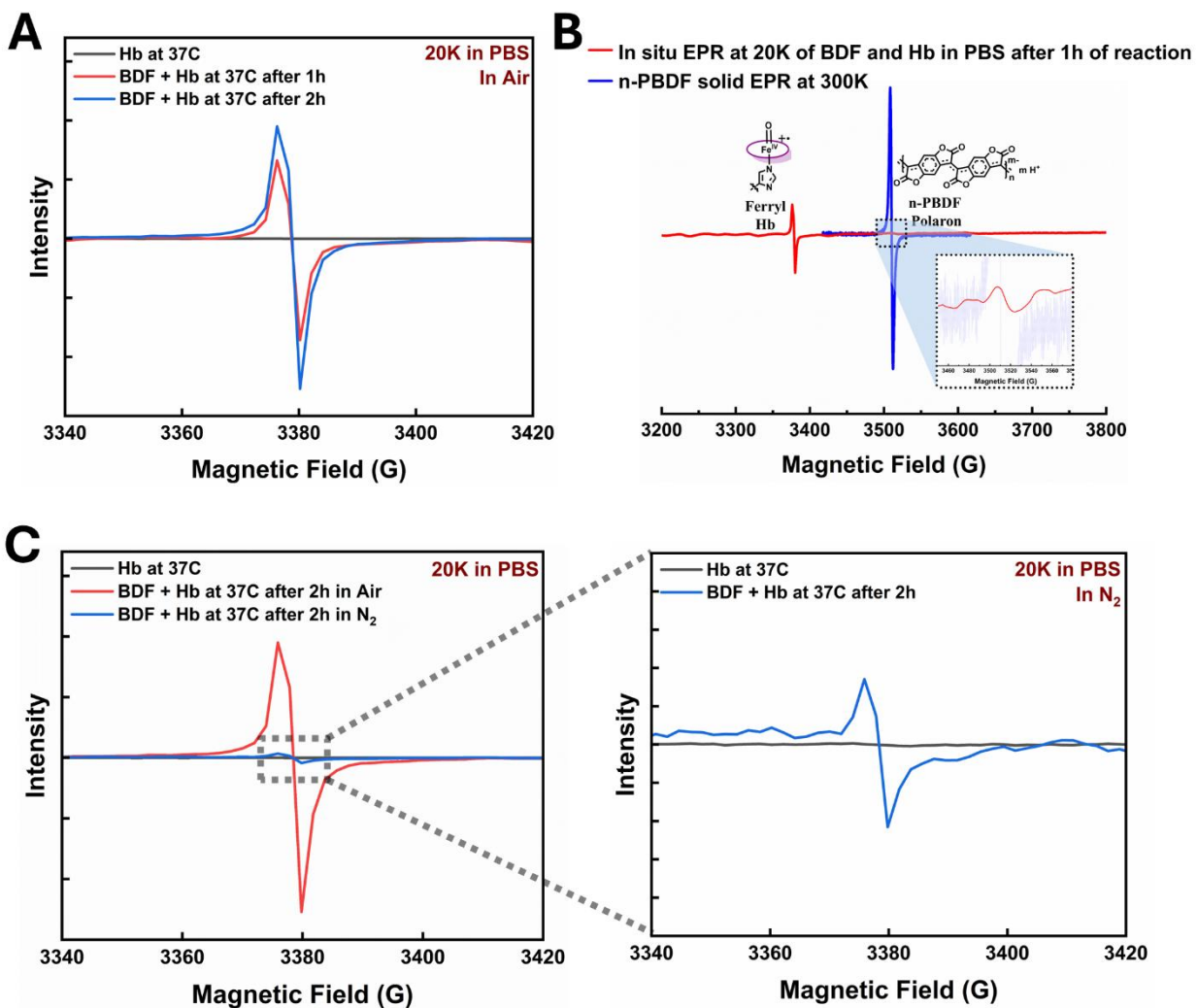


Figure S22. (A) *In situ* EPR spectrum of a frozen sample of BDF and Hb mixture in air after different reaction times, (B) Comparison of the *in situ* EPR spectrum of a frozen sample of the BDF and Hb mixture in air after one hour of reaction with an EPR spectrum of a solid n-PBDF sample, and (C) Comparison of the *in situ* EPR spectrum of a frozen sample of BDF and Hb mixture in air vs. inert-nitrogen environment after two hours of reaction (zoomed spectra in the case of nitrogen environment).

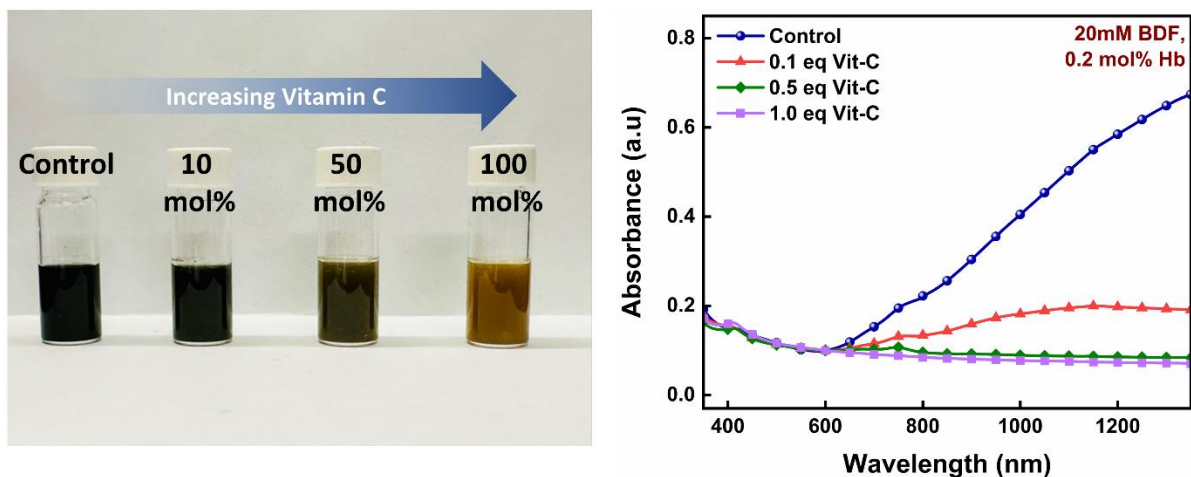


Figure S23. Polymerization of n-PBDF in the presence of ascorbate (vitamin C) and its corresponding UV-Vis-NIR spectrum.

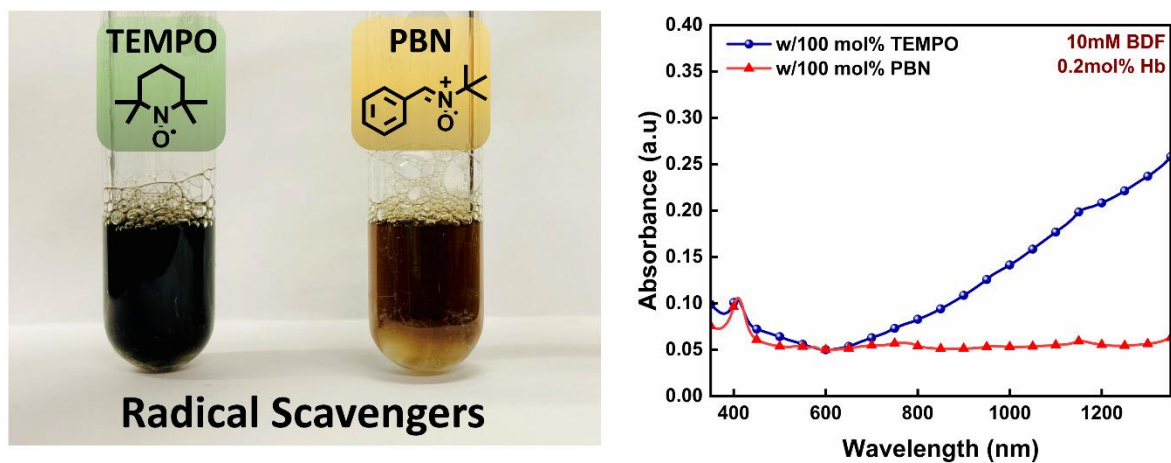


Figure S24. Polymerization of n-PBDF in the presence of radical scavengers and its corresponding solution UV-Vis-NIR spectrum.

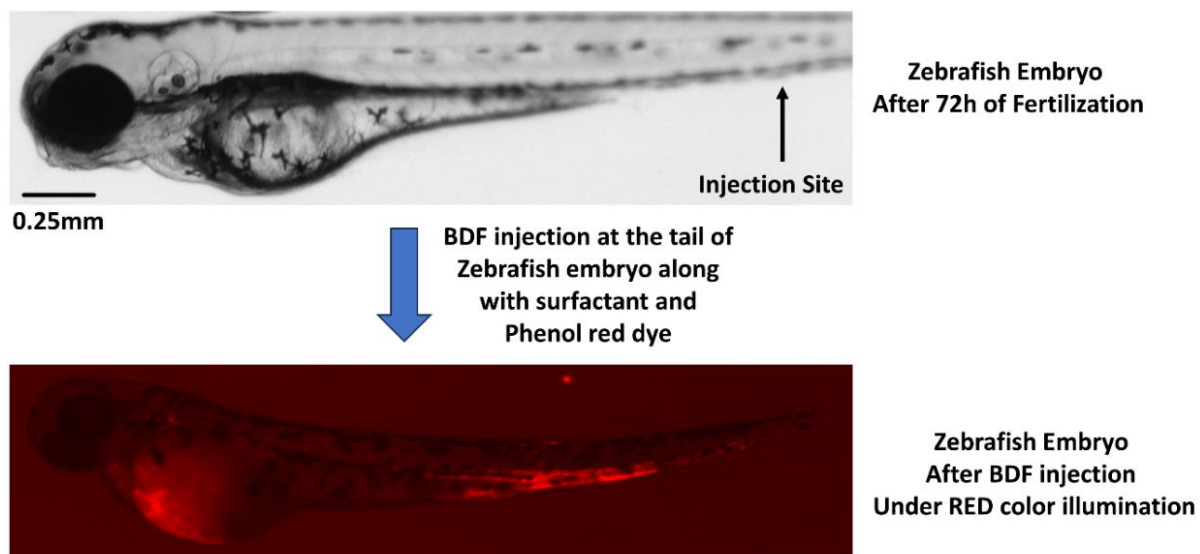


Figure S25. Injection site and injection confirmation in zebrafish embryos.

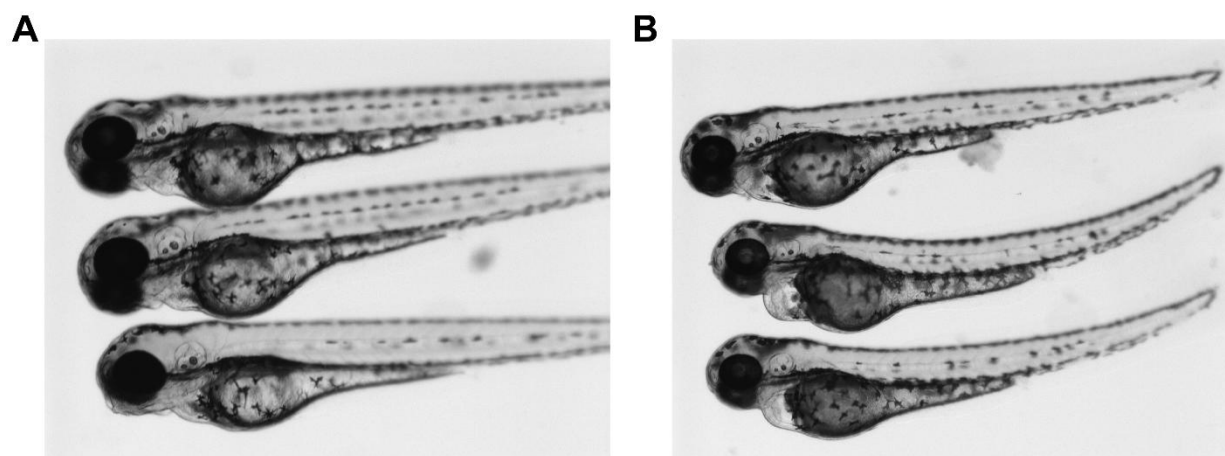


Figure S26. *In-vivo* formation of n-PBDF inside zebrafish embryo after 24h incubation at 34°C (A) control, and (B) 10mM BDF injection.

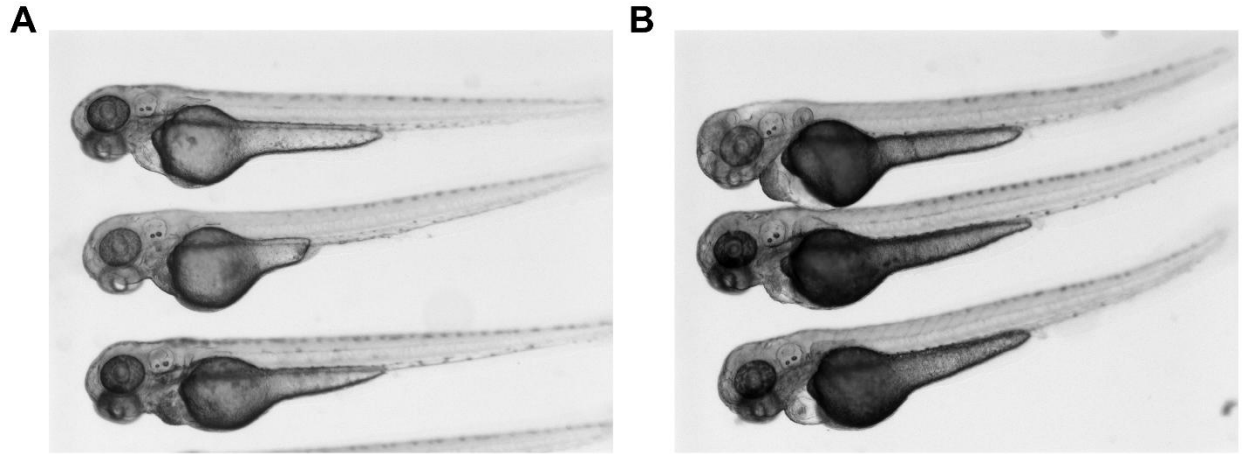


Figure S27. *In-vivo* formation of n-PBDF inside zebrafish embryo with PTU treatment after 24h incubation at 34°C (A) control, and (B) 10mM BDF injection.

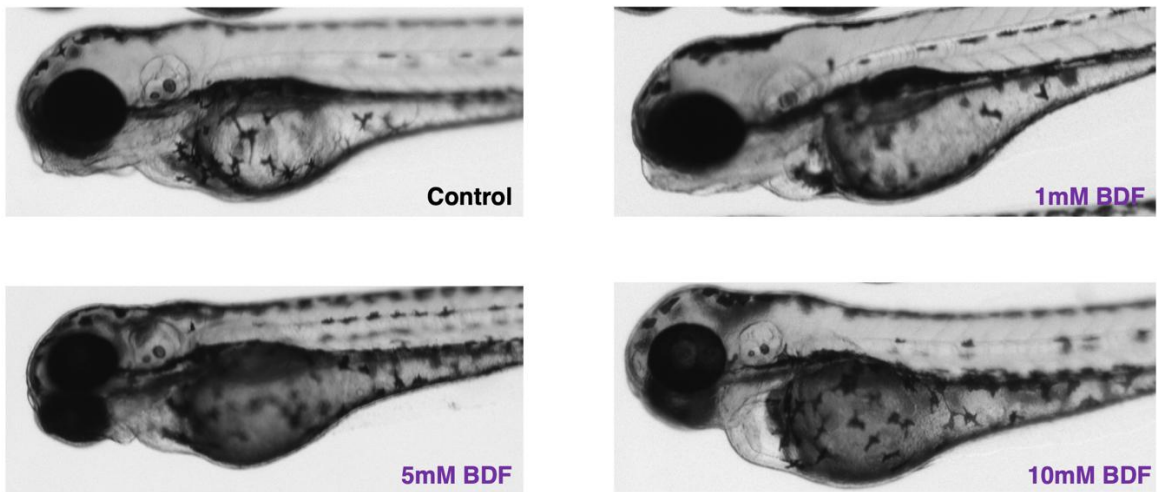


Figure S28. *In-vivo* formation of n-PBDF inside zebrafish embryo with varying BDF injection after 24h incubation at 34°C.

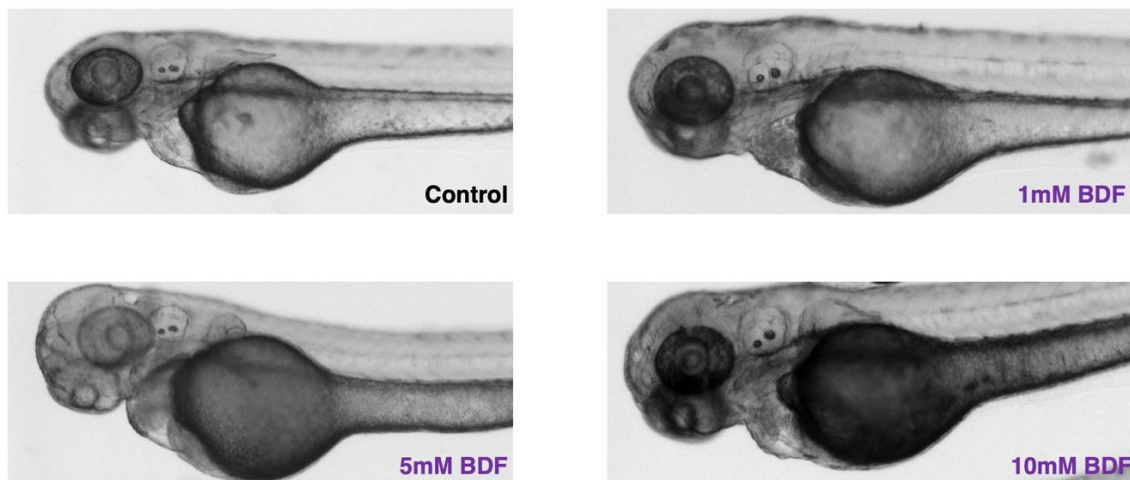


Figure S29. *In-vivo* formation of n-PBDF inside zebrafish embryo with varying BDF injection after 24h incubation at 34°C and with PTU treatment throughout the experiment.

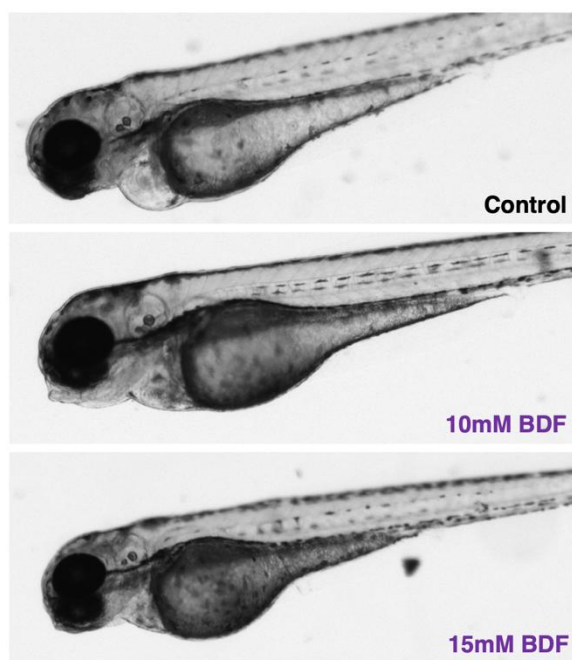
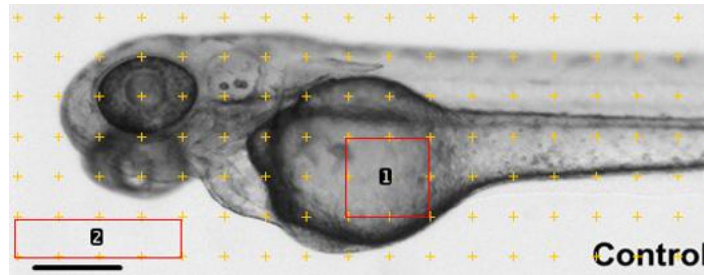


Figure S30. *In-vivo* formation of n-PBDF inside zebrafish embryo with varying BDF injection after 24h incubation at 34°C and with PTU treatment only until the BDF injection.

Quantification of Brightness of Yolk in Zebrafish Embryos:

All brightness measurements were done in ImageJ 1.54g (Wayne Rasband and contributors, National Institute of Health, USA). Two boxes of the same areas are drawn for each image of zebrafish embryos. The first box is outside of the fish (this is a control of brightfield imaging, capturing the difference in total light), and the second box is in the core of the yolk.

- Sample quantification:
 - Control zebrafish embryos/PTU-treated

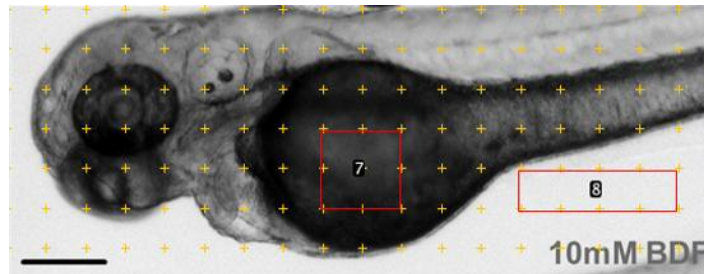


Box number 1: Area = 0.397, Mean light: 144.702, Mean/Area = 364.488

Box number 2: Area = 0.384, Mean light: 234.794, Mean/Area = 611.442

Relative Yolk Intensity $I_{\text{control}} = 364.488/611.442 = 0.596$

- 10mM BDF injection/PTU-treated



Box number 1: Area = 0.391, Mean light: 48.567, Mean/Area = 124.212

Box number 2: Area = 0.407, Mean light: 233.047, Mean/Area = 572.597

Relative Yolk Intensity $I_{10\text{mMPTU}} = 124.212/572.597 = 0.217$

$$\text{Normalized Brightness (yolk) } IN_{10\text{mMPTU}} = \frac{I_{10\text{mMPTU}}}{I_{\text{control}}} \times 100 = 36.4\%$$

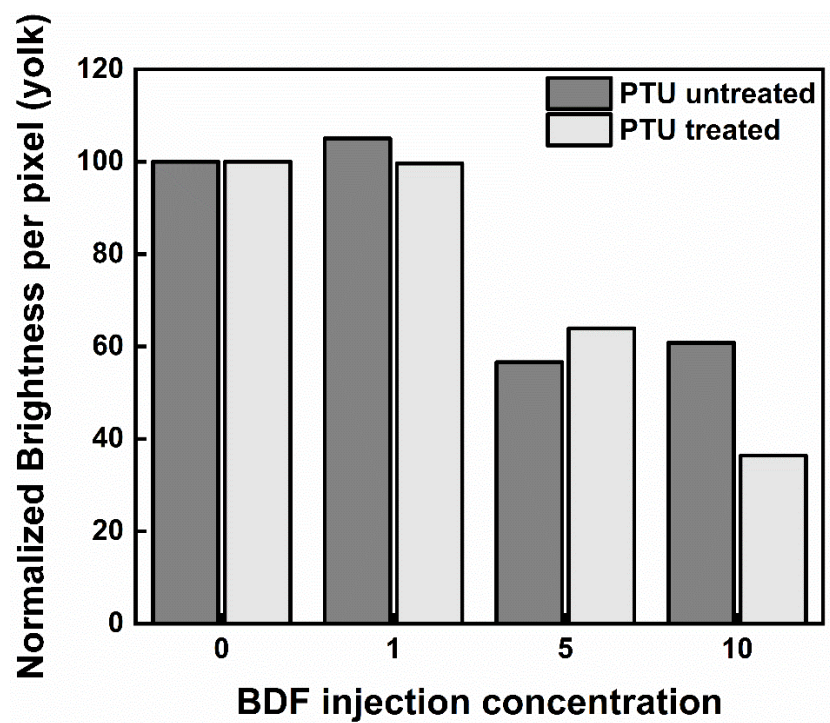


Figure S31. Normalized Brightness change in zebrafish yolk with BDF injection with respect to the control.

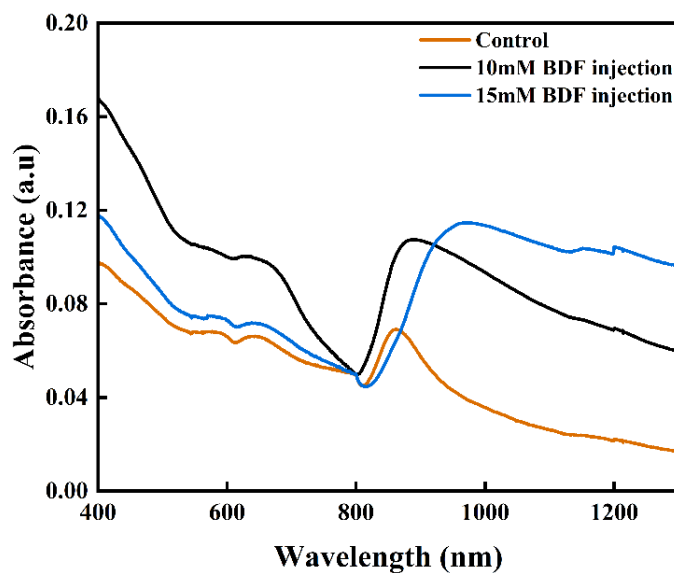


Figure S32. UV-Vis-NIR spectrum of zebrafish embryos after 24h incubation at 34 °C with different BDF injection concentrations.

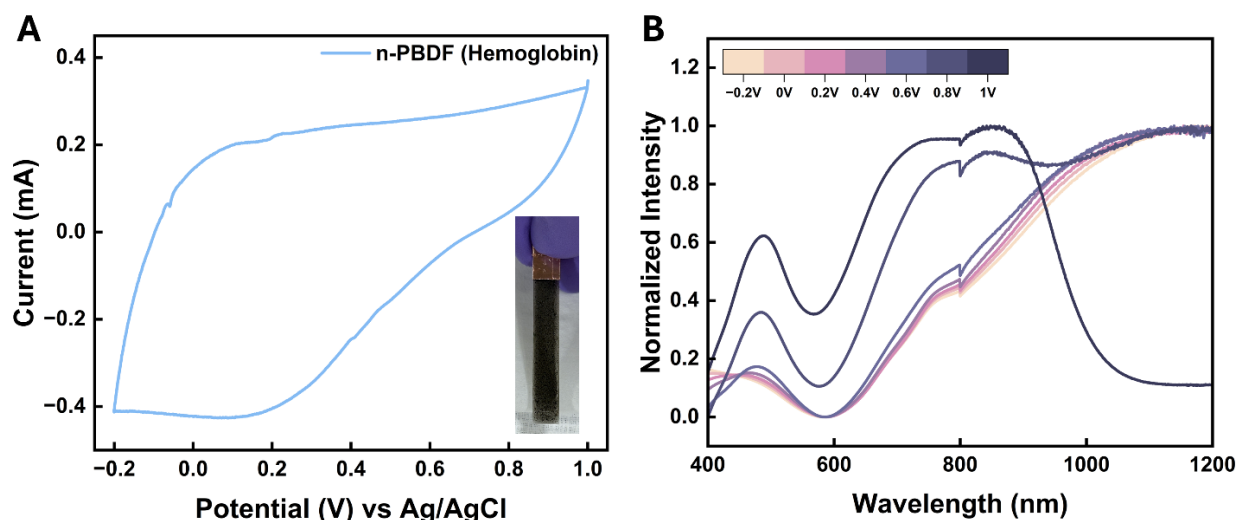


Figure S33. Electrochemical properties of n-PBDF polymerized with hemoglobin without purification, (A) Cyclic voltammogram of the n-PBDF film in 0.2M TBA-TFSI in propylene carbonate (PC) (Inset: drop-casted n-PBDF film on ITO substrate and vacuum dried.), and (B) In situ electrochemical UV-Vis-NIR absorption spectroscopy of the n-PBDF film.

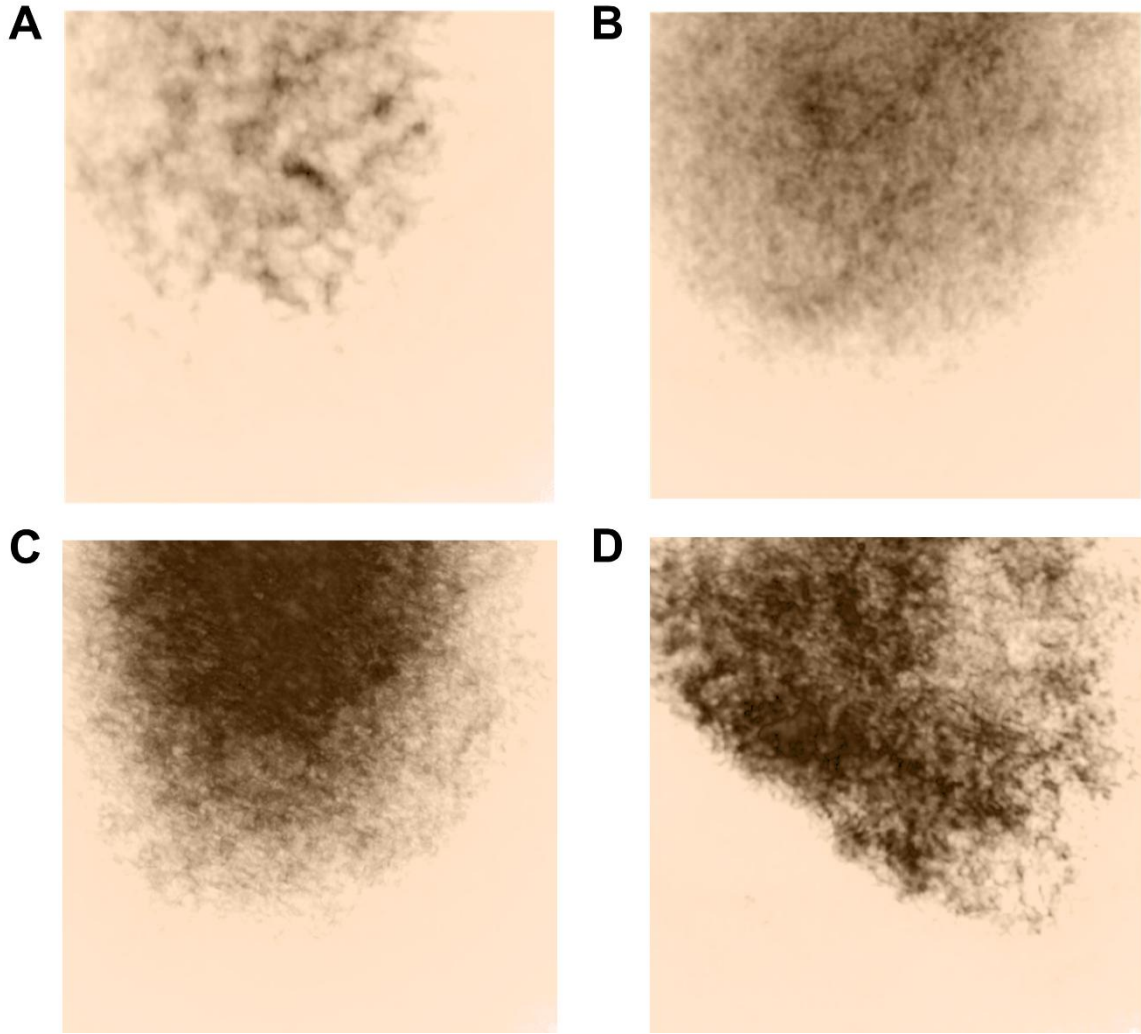


Figure S34. Absorption image of zebrafish embryo's yolk at 960 nm after 24h incubation at 34 °C with different BDF injection concentrations, (A) Control, (B) 10mM BDF, (C) 15mM BDF, and (D) 15mM BDF with PTU treatment.

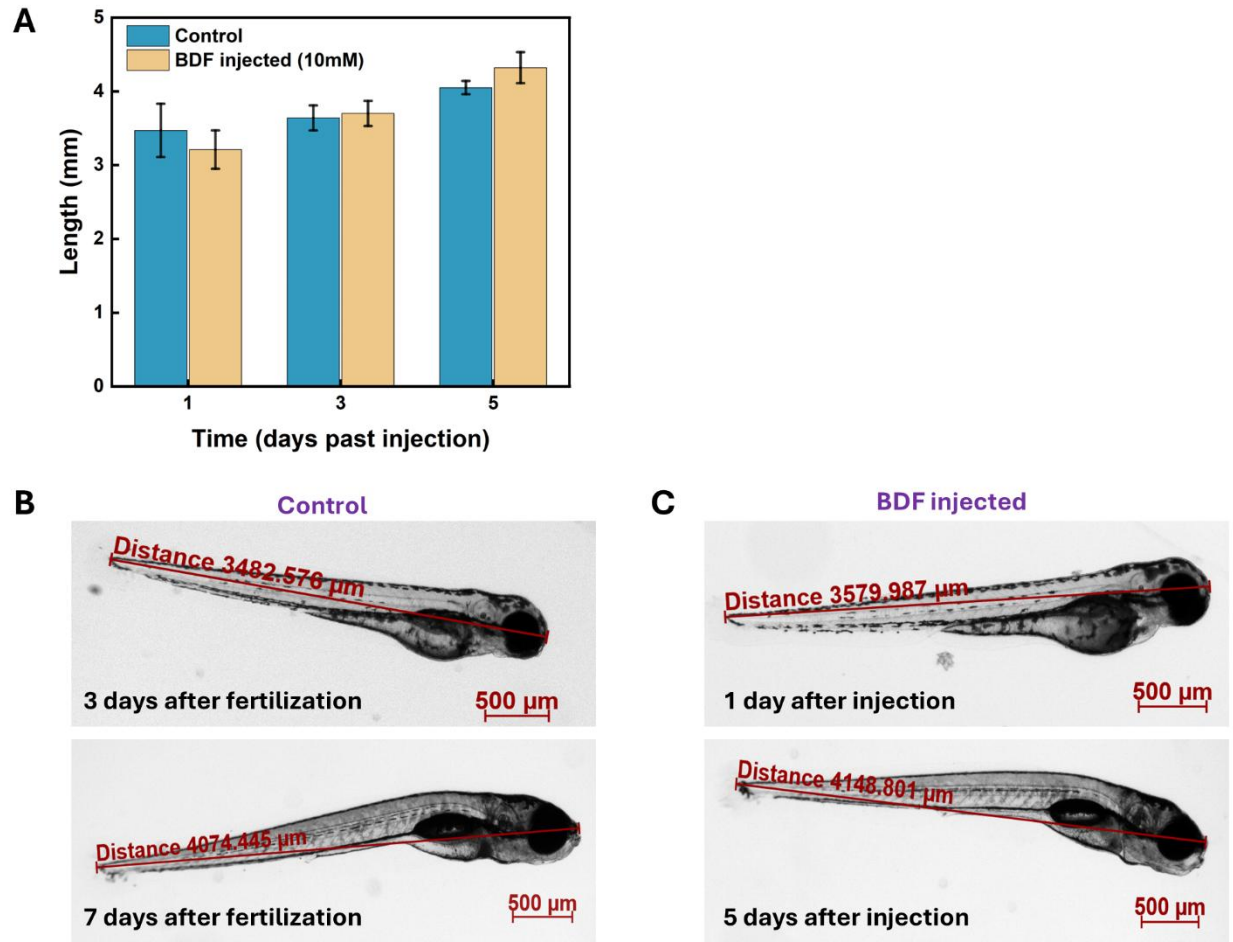


Figure S35. Growth of zebrafish larvae. (A) Quantifying the length of zebrafish larvae over several days with and without BDF injection, a representative length quantification of (B) control and (C) BDF-injected larvae.

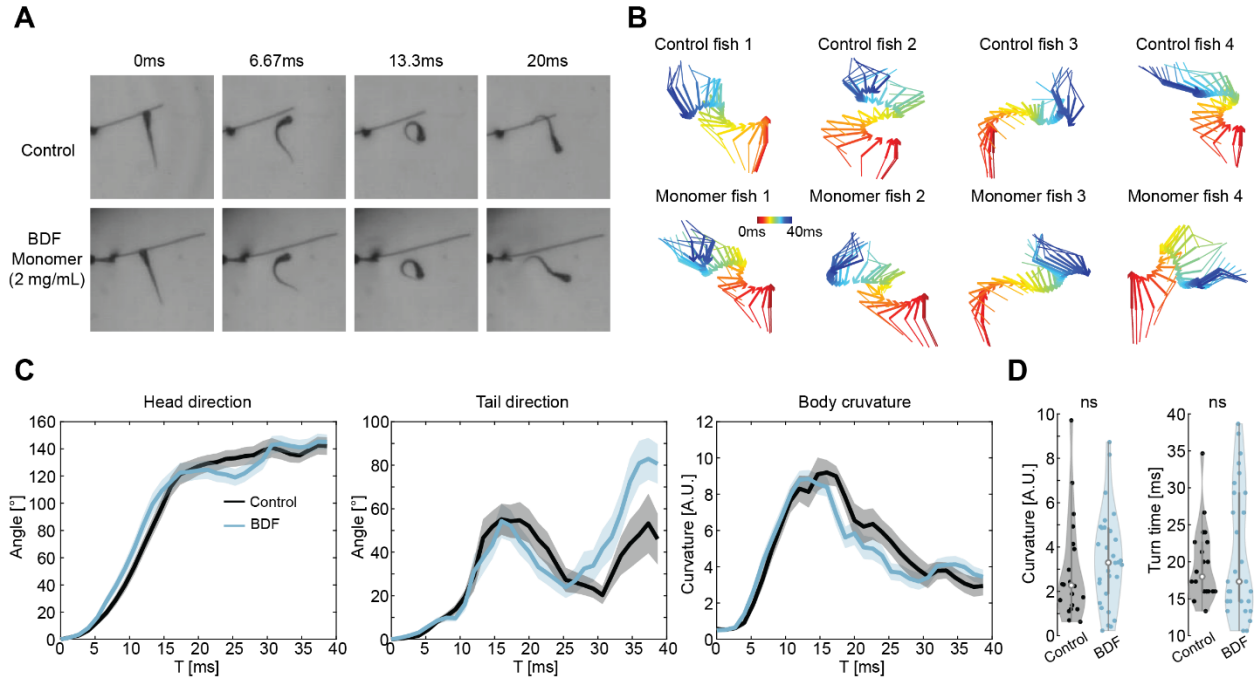


Figure S36. C-turn behavior of zebrafish larvae. **(A)** Example frames showing the c-turn behavior of larvae injected with PBS (control) and BDF upon a tactile stimulus on the head. **(B)** Example tracked movement of the larvae's c-turn behavior; the arrows denote the head direction. **(C)** The change in head direction, tail direction, and body curvature as a function of time during the c-turn behavior of the control (black) and BDF-injected fishes (n = 20 control fishes and 30 BDF-injected fishes, mean $\pm$ sem). **(D)** The maximum body curvature (left) and turn-around time (right) show no difference between control and BDF-injected fish.

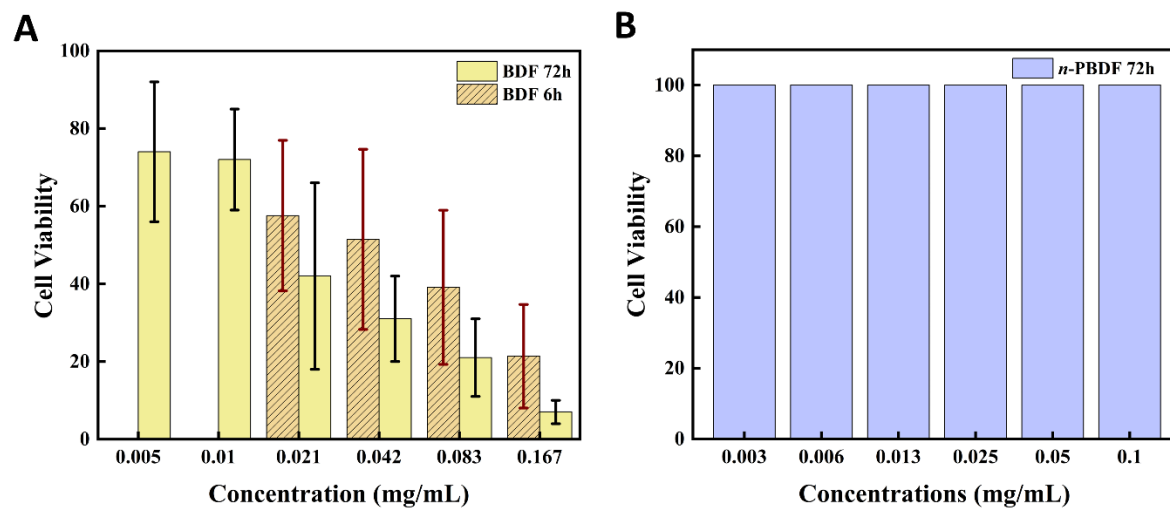


Figure S37. SRB cytotoxicity data of (A) BDF monomer, and (B) n-PBDF polymer.



Purdue Veterinary Clinical Pathology Laboratory

Purdue University College of Veterinary Medicine
625 Harrison Street, Rm G351
West Lafayette, IN 47907

Phone: (765)494-7563 Fax: (765)494-8640 Lab Dir: John Christian, DVM, PhD



Clinician: Robyn McCain

Purdue Research
00000

Sex: M

Age: 1mo

DOB: 3/8/2024

Species: Mouse

Breed:

Patient name: Knipp, M2 Monomer

Sample ID: C240409014

Hospital Case#: RS20240112

Encounter ID

Collection Date: 4/9/2024 9:15 AM

Received Date: 4/9/2024 9:18 AM

Small Animal Panel 2

Slight Hemolysis

Plasma

MCMLS			Ref. Range/Males
GLU	H	198	90-192 mg/dL
BUN		21	19-29 mg/dL
CREA		<0.20	0.20-0.80 mg/dL
PHOS		8.6	6.1-10.0 mg/dL
CA	H	10.3	5.9-9.4 mg/dL
NA		139	124-174 mmol/L
K		5.1	4.6-8.0 mmol/L
CL		110	92-120 mmol/L
CO2		23	mmol/L
AGAP		11.1	mmol/L
TP		4.8	3.6-6.6 g/dL
ALB	L	2.3	2.5-4.8 g/dL
GLOB	H	2.5	0.0-0.6 g/dL
A/G		0.9	
ALTv		33	28-132 U/L
ALKP		136	62-209 IU/L
GGT		<10	IU/L
TBIL		0.30	mg/dL
CHOL	H	165	36-96 mg/dL
AMY		2203	1691-3615 IU/L
LIPA		811	IU/L

Figure S38. Clinical blood report of the mice after 1 day of subcutaneous injection of 0.1 mL solution of 10mM BDF in PBS with 1wt% TPGS-750-M and 5% DMSO.



Purdue Veterinary Clinical Pathology Laboratory

Purdue University College of Veterinary Medicine
625 Harrison Street, Rm G351
West Lafayette, IN 47907

Phone: (765)494-7563 Fax: (765)494-8640 Lab Dir: John Christian, DVM, PhD



Clinician: Robyn McCam

Purdue Research
00000

Sex: M

Age: 1mo

DOB: 3/8/2024

Species: Mouse

Breed:

Patient name: Knipp, M3 Monomer

Sample ID: C240411018

Hospital Case#: RS20240115

Encounter ID

Collection Date: 4/11/2024 9:38 AM

Received Date: 4/11/2024 9:48 AM

SA aCBC

These results were generated by the Siemens Advia 2120i Hematology analyzer and have not been verified with slide review. They should be considered preliminary until confirmed by trained laboratory testing personnel.

CPTMS		Ref. Range/Males
TP (R)	5.3	g/dL
RBC	7.49	M/uL
HCT	40.0	%
Manual hematocrit used		
HGB	13.1	g/dL
MCV	57.2	fL
MCHC	32.8	g/dL
RDW	13.5	%
WBC	7.4	K/uL
PLAT	2037	K/uL
MPV	6.9	fL
RETIC #	304.1	K/uL

Manual Diff (Advia)

CPTAB		Ref. Range/Males
WBC	7.4	K/uL
SEG	1.0	K/uL
LYMPH	5.1	K/uL
MONO	0.44	K/uL
EOS	0.81	K/uL
POLYCHROMASIA	2+	
R. LYMPHS	Noted	
PLAT	2037 *clumped*	K/uL

Platelet clumping is present. An accurate platelet count is not possible by machine or blood smear. The number reported here represents the machine's count of non-clumped platelets. The true count should be higher, the amount higher depending on the size and number of clumps. The reported platelet count from clumped samples is NOT a valid metric for following platelet counts sequentially.

PLT EST Increase

Small Animal Panel 2

CPTMP			Ref. Range/Males
GLU	H	196	90-192 mg/dL
BUN		22	19-29 mg/dL
CREA		<0.20	0.20-0.80 mg/dL
PHOS		8.3	6.1-10.0 mg/dL
CA	H	10.1	5.9-9.4 mg/dL
NA		140	124-174 mmol/L
K	L	4.3	4.6-8.0 mmol/L
CL		113	92-120 mmol/L
CO2		17	mmol/L
AGAP		14.3	mmol/L
TP		4.8	3.6-6.6 g/dL
ALB	L	2.3	2.5-4.8 g/dL
GLOB	H	2.5	0.0-0.6 g/dL
A/G		0.9	
ALTv	L	25	28-132 U/L
ALKP		110	62-209 IU/L
GGT		<10	IU/L
TBIL		0.30	mg/dL
CHOL	H	133	36-96 mg/dL
AMY		2613	1691-3615 IU/L
LIPA		756	IU/L

Figure S39. Clinical blood report of the mice after 3 days of subcutaneous injection of 0.1 mL solution of 10mM BDF in PBS with 1wt% TPGS-750-M and 5% DMSO.



Purdue Veterinary Clinical Pathology Laboratory

Purdue University College of Veterinary Medicine
625 Harrison Street, Rm G351
West Lafayette, IN 47907

Phone: (765)494-7563 Fax: (765)494-8640 Lab Dir: John Christian, DVM, PhD



Clinician: Robyn McCain

Purdue Research
(00000)

Sex: M

Age: 1mo

DOB: 3/8/2024

Species: Mouse

Breed:

Patient name: Knipp, M4 Monomer

Sample ID: C240415020

Hospital Case#: RS20240119

Encounter ID

Collection Date: 4/15/2024 9:04 AM

Received Date: 4/15/2024 9:17 AM

SA aCBC

These results were generated by the Siemens Advia 2120i Hematology analyzer and have not been verified with slide review. They should be considered preliminary until confirmed by trained laboratory testing personnel.

AHMLT		Ref. Range/Males
TP (R)	5.2	g/dL
RBC	8.07	M/uL
HCT	40.0	%
Manual hematocrit used		
HGB	13.1	g/dL
MCV	53.2	fL
MCHC	32.8	g/dL
RDW	14.2	%
WBC	5.4	K/uL
PLAT	1280	K/uL
MPV	7.4	fL
RETIC #	259.7	K/uL

Manual Diff (Advia)

CPTMS		Ref. Range/Males
WBC	5.4	K/uL
SEG	0.4	K/uL
LYMPH	4.9	K/uL
MONO	0.05	K/uL
POIK	1+	
POLYCHROMASIA	2+	
R. LYMPHS	Noted	
PLAT	1280 *clumped*	K/uL

Platelet clumping is present. An accurate platelet count is not possible by machine or blood smear. The number reported here represents the machine's count of non-clumped platelets. The true count should be higher, the amount higher depending on the size and number of clumps. The reported platelet count from clumped samples is NOT a valid metric for following platelet counts sequentially.

PLT EST Increase
PLTS COMMENT: ;
Enlarged Platelets Noted

Small Animal Panel 2

Plasma

CPTNR		Ref. Range/Males
GLU	H 194	90-192 mg/dL
BUN	21	19-29 mg/dL
CREA	<0.20	0.20-0.80 mg/dL
PHOS	7.1	6.1-10.0 mg/dL
CA	H 9.9	5.9-9.4 mg/dL
NA	141	124-174 mmol/L
K	L 3.6	4.6-8.0 mmol/L
CL	114	92-120 mmol/L
CO2	16	mmol/L
AGAP	14.6	mmol/L
TP	4.8	3.6-6.6 g/dL
ALB	L 2.3	2.5-4.8 g/dL
GLOB	H 2.5	0.0-0.6 g/dL
A/G	0.9	
ALTv	31	28-132 U/L
ALKP	110	62-209 IU/L
GGT	<10	IU/L
TBIL	0.20	mg/dL
CHOL	H 113	36-96 mg/dL
AMY	2632	1691-3615 IU/L
LIPA	951	IU/L

Figure S40. Clinical blood report of the mice after 7 days of subcutaneous injection of 0.1 mL solution of 10mM BDF in PBS with 1wt% TPGS-750-M and 5% DMSO.



Purdue Veterinary Clinical Pathology Laboratory

Purdue University College of Veterinary Medicine
625 Harrison Street, Rm G351
West Lafayette, IN 47907

Phone: (765)494-7563 Fax: (765)494-8640 Lab Dir: John Christian, DVM, PhD



Clinician: Robyn McCain

Purdue Research
00000

Sex: M

Age: 20d

DOB: 3/20/2024

Species: Mouse

Breed:

Patient name: Knipp, M12 M+C

Sample ID: C240409018

Hospital Case#: RS20240114

Encounter ID

Collection Date: 4/9/2024 9:24 AM

Received Date: 4/9/2024 9:26 AM

Small Animal Panel 2

Slight Hemolysis

Plasma

MCMLS			Ref. Range/Males
GLU	H	200	90-192 mg/dL
BUN	L	16	19-29 mg/dL
CREA		0.20	0.20-0.80 mg/dL
PHOS		8.1	6.1-10.0 mg/dL
CA	H	11.1	5.9-9.4 mg/dL
NA		141	124-174 mmol/L
K	L	3.9	4.6-8.0 mmol/L
CL		110	92-120 mmol/L
CO2		20	mmol/L
AGAP		14.9	mmol/L
TP		5.0	3.6-6.6 g/dL
ALB	L	2.3	2.5-4.8 g/dL
GLOB	H	2.7	0.0-0.6 g/dL
A/G		0.9	
ALTv		34	28-132 U/L
ALKP		107	62-209 IU/L
GGT		<10	IU/L
TBIL		0.20	mg/dL
CHOL	H	114	36-96 mg/dL
AMY		2296	1691-3615 IU/L
LIPA		706	IU/L

Figure S41. Clinical blood report of the mice after 1 day of subcutaneous injection of 0.1 mL solution of 10mM BDF and 20 μ M Hb in PBS with 1wt% TPGS-750-M and 5% DMSO.



Purdue Veterinary Clinical Pathology Laboratory

Purdue University College of Veterinary Medicine
625 Harrison Street, Rm G351
West Lafayette, IN 47907

Phone: (765)494-7563 Fax: (765)494-8640 Lab Dir: John Christian, DVM, PhD



Clinician: Robyn McCain

Purdue Research
00000

Sex: M

Age: 1mo

DOB: 3/8/2024

Species: Mouse

Breed:

Patient name: Knipp, M13 M&C

Sample ID: C240411020

Hospital Case#: RS20240117

Encounter ID

Collection Date: 4/11/2024 9:48 AM

Received Date: 4/11/2024 9:50 AM

Small Animal Panel 2

Slight Hemolysis, Slight Lipemia

CPTMP		Ref. Range/Males
GLU	165	90-192 mg/dL
BUN	20	19-29 mg/dL
CREA	<0.20	0.20-0.80 mg/dL
PHOS	8.1	6.1-10.0 mg/dL
CA	H 10.5	5.9-9.4 mg/dL
NA	142	124-174 mmol/L
K	L 4.1	4.6-8.0 mmol/L
CL	111	92-120 mmol/L
CO2	24	mmol/L
AGAP	11.1	mmol/L
TP	5.1	3.6-6.6 g/dL
ALB	L 2.4	2.5-4.8 g/dL
GLOB	H 2.7	0.0-0.6 g/dL
A/G	0.9	
ALTv	30	28-132 U/L
ALKP	119	62-209 IU/L
GGT	<10	IU/L
TBIL	0.20	mg/dL
CHOL	H 122	36-96 mg/dL
AMY	2559	1691-3615 IU/L
LIPA	931	IU/L

Figure S42. Clinical blood report of the mice after 3 days of subcutaneous injection of 0.1 mL solution of 10mM BDF and 20 μ M Hb in PBS with 1wt% TPGS-750-M and 5% DMSO.



Purdue Veterinary Clinical Pathology Laboratory

Purdue University College of Veterinary Medicine
625 Harrison Street, Rm G351
West Lafayette, IN 47907

Phone: (765)494-7563 Fax: (765)494-8640 Lab Dir: John Christian, DVM, PhD



Clinician: Robyn McCain

Purdue Research
00000

Sex: M

Age: 1mo

DOB: 3/8/2024

Species: Mouse

Breed:

Patient name: Knipp, M14 M+C

Sample ID: C240415022

Hospital Case#: RS20240121

Encounter ID

Collection Date: 4/15/2024 9:18 AM

Received Date: 4/15/2024 9:19 AM

SA aCBC

These results were generated by the Siemens Advia 2120i Hematology analyzer and have not been verified with slide review. They should be considered preliminary until confirmed by trained laboratory testing personnel.

		Ref. Range/Males
AHMLT		
TP (R)	5.3	g/dL
RBC	7.90	M/uL
HCT	42.1	%
HGB	13.3	g/dL
MCV	53.2	fL
MCHC	31.5	g/dL
RDW	13.9	%
WBC	7.6	K/uL
PLAT	1313	K/uL
MPV	7.2	fL
RETIC #	225.2	K/uL

Manual Diff (Advia)

		Ref. Range/Males
CPTMS		
WBC	7.6	K/uL
SEG	1.0	K/uL
LYMPH	6.3	K/uL
MONO	0.23	K/uL
EOS	0.08	K/uL
POIK	1+	
POLYCHROMASIA	2+	
R. LYMPHS	Noted	
PLAT	1313 *clumped*	K/uL

Platelet clumping is present. An accurate platelet count is not possible by machine or blood smear. The number reported here represents the machine's count of non-clumped platelets. The true count should be higher, the amount higher depending on the size and number of clumps. The reported platelet count from clumped samples is NOT a valid metric for following platelet counts sequentially.

PLT EST Increase

PLTS COMMENT: :
Enlarged Platelets Noted

Small Animal Panel 2

Plasma, Slight Lipemia

			Ref. Range/Males
CPTNR			
GLU	H	198	90-192 mg/dL
BUN		20	19-29 mg/dL
CREA		<0.20	0.20-0.80 mg/dL
PHOS		8.3	6.1-10.0 mg/dL
CA	H	9.8	5.9-9.4 mg/dL
NA		140	124-174 mmol/L
K	L	3.8	4.6-8.0 mmol/L
CL		112	92-120 mmol/L
CO2		19	mmol/L
AGAP		12.8	mmol/L
TP		4.8	3.6-6.6 g/dL
ALB	L	2.3	2.5-4.8 g/dL
GLOB	H	2.5	0.0-0.6 g/dL
A/G		0.9	
ALTv		32	28-132 U/L
ALKP		112	62-209 IU/L
GGT		<10	IU/L
TBIL		0.30	mg/dL
CHOL	H	126	36-96 mg/dL
AMY		2634	1691-3615 IU/L
LIPA		995	IU/L

Figure S43. Clinical blood report of the mice after 7 days of subcutaneous injection of 0.1 mL solution of 10mM BDF and 20µM Hb in PBS with 1wt% TPGS-750-M and 5% DMSO.



Purdue Veterinary Clinical Pathology Laboratory

Purdue University College of Veterinary Medicine
625 Harrison Street, Rm G351
West Lafayette, IN 47907

Phone: (765)494-7563 Fax: (765)494-8640 Lab Dir: John Christian, DVM, PhD



Clinician: Robyn McCain

Purdue Research
00000

Sex: M

Age: 1mo

DOB: 3/8/2024

Species: Mouse

Breed:

Patient name: RS20240113, M7

Sample ID: C240409017

Hospital Case#: RS20240113

Encounter ID

Collection Date: 4/9/2024 9:23 AM

Received Date: 4/9/2024 9:25 AM

Small Animal Panel 2

Hemolysis results in a positive bias for total bilirubin, total protein, albumin, potassium, phosphorus, and a negative bias for glucose.

Marked Hemolysis

Plasma

MCMLS		Ref. Range/Males
GLU	H 200	90-192 mg/dL
BUN	21	19-29 mg/dL
CREA	<0.20	0.20-0.80 mg/dL
PHOS	H 10.5	6.1-10.0 mg/dL
CA	H 9.5	5.9-9.4 mg/dL
NA	137	124-174 mmol/L
K	7.9	4.6-8.0 mmol/L
CL	109	92-120 mmol/L
CO2	20	mmol/L
AGAP	15.9	mmol/L
TP	5.1	3.6-6.6 g/dL
ALB	2.6	2.5-4.8 g/dL
GLOB	H 2.5	0.0-0.6 g/dL
A/G	1.0	
ALTv	87	28-132 U/L
ALKP	110	62-209 IU/L
GGT	24	IU/L
TBIL	1.40	mg/dL
CHOL	H 122	36-96 mg/dL
AMY	2212	1691-3615 IU/L
LIPA	881	IU/L

Figure S44. Clinical blood report of the mice after 1 day of subcutaneous injection of 0.1 mL n-PBDF solution polymerized from 10mM BDF and 20 μ M Hb in PBS with 1wt% TPGS-750-M and 5% DMSO.



Purdue Veterinary Clinical Pathology Laboratory

Purdue University College of Veterinary Medicine
625 Harrison Street, Rm G351
West Lafayette, IN 47907

Phone: (765)494-7563 Fax: (765)494-8640 Lab Dir: John Christian, DVM, PhD



Clinician: Robyn McCain

Purdue Research
00000

Sex: M

Age: 1mo

DOB: 3/8/2024

Species: Mouse

Breed:

Patient name: Knipp, M8 Polymer

Sample ID: C240411019

Hospital Case#: RS20240116

Encounter ID

Collection Date: 4/11/2024 9:47 AM

Received Date: 4/11/2024 9:49 AM

SA aCBC

These results were generated by the Siemens Advia 2120i Hematology analyzer and have not been verified with slide review. They should be considered preliminary until confirmed by trained laboratory testing personnel.

CPTMS		Ref. Range/Males
TP (R)	5.0	g/dL
RBC	8.23	M/uL
HCT	43.0	%
Manual hematocrit used		
HGB	13.8	g/dL
MCV	54.3	fL
MCHC	32.1	g/dL
RDW	12.7	%
WBC	6.2	K/uL
PLAT	1390	K/uL
MPV	5.8	fL
RETIC #	211.0	K/uL

Manual Diff (Advia)

CPTMS		Ref. Range/Males
WBC	6.2	K/uL
SEG	1.4	K/uL
LYMPH	4.6	K/uL
MONO	0.06	K/uL
EOS	0.06	K/uL
POIK	1+	
POLYCHROMASIA	1+	
R. LYMPHS	Noted	
PLAT	1390 *clumped*	K/uL

Platelet clumping is present. An accurate platelet count is not possible by machine or blood smear. The number reported here represents the machine's count of non-clumped platelets. The true count should be higher, the amount higher depending on the size and number of clumps. The reported platelet count from clumped samples is NOT a valid metric for following platelet counts sequentially.

PLT EST Increase

_Small Animal Panel 2

CPTMP		Ref. Range/Males
GLU	178	90-192 mg/dL
BUN	19	19-29 mg/dL
CREA	0.20	0.20-0.80 mg/dL
PHOS	8.9	6.1-10.0 mg/dL
CA	H 9.9	5.9-9.4 mg/dL
NA	138	124-174 mmol/L
K	4.6	4.6-8.0 mmol/L
CL	112	92-120 mmol/L
CO2	16	mmol/L
AGAP	14.6	mmol/L
TP	4.7	3.6-6.6 g/dL
ALB	L 2.1	2.5-4.8 g/dL
GLOB	H 2.6	0.0-0.6 g/dL
A/G	0.8	
ALTv	L 27	28-132 U/L
ALKP	95	62-209 IU/L
GGT	<10	IU/L
TBIL	0.20	mg/dL
CHOL	H 118	36-96 mg/dL
AMY	2317	1691-3615 IU/L
LIPA	809	IU/L

Figure S45. Clinical blood report of the mice after 3 days of subcutaneous injection of 0.1 mL n-PBDF solution polymerized from 10mM BDF and 20µM Hb in PBS with 1wt% TPGS-750-M and 5% DMSO.



Purdue Veterinary Clinical Pathology Laboratory

Purdue University College of Veterinary Medicine
625 Harrison Street, Rm G351
West Lafayette, IN 47907

Phone: (765)494-7563 Fax: (765)494-8640 Lab Dir: John Christian, DVM, PhD



Clinician: Robyn McCain

Purdue Research
00000

Sex: M

Age: Imo

DOB: 3/8/2024

Species: Mouse

Breed:

Patient name: Knipp, M9 Polymer

Sample ID: C240415021

Hospital Case#: RS20240120

Encounter ID

Collection Date: 4/15/2024 9:16 AM

Received Date: 4/15/2024 9:18 AM

SA aCBC

These results were generated by the Siemens Advia 2120i Hematology analyzer and have not been verified with slide review. They should be considered preliminary until confirmed by trained laboratory testing personnel.

		Ref. Range/Males
AHMLT		
TP (R)	5.0	g/dL
RBC	7.68	M/uL
HCT	40.7	%
HGB	12.8	g/dL
MCV	52.9	fL
MCHC	31.5	g/dL
RDW	13.5	%
WBC	6.2	K/uL
PLAT	969	K/uL
MPV	7.0	fL
RETIC #	259.9	K/uL

Manual Diff (Advia)

		Ref. Range/Males
CPTMS		
WBC	6.2	K/uL
SEG	0.6	K/uL
LYMPH	5.5	K/uL
MONO	0.06	K/uL
POIK	1+	
POLYCHROMASIA	2+	
R. LYMPHS	Noted	
PLAT	969 *clumped*	K/uL

Platelet clumping is present. An accurate platelet count is not possible by machine or blood smear. The number reported here represents the machine's count of non-clumped platelets. The true count should be higher, the amount higher depending on the size and number of clumps. The reported platelet count from clumped samples is NOT a valid metric for following platelet counts sequentially.

PLT EST: Increase
PLTS COMMENT: ;
Enlarged Platelets Noted

Small Animal Panel 2

Plasma, Slight Hemolysis, Slight Lipemia

		Ref. Range/Males
CPTNR		
GLU	189	90-192 mg/dL
BUN	L 18	19-29 mg/dL
CREA	0.20	0.20-0.80 mg/dL
PHOS	7.0	6.1-10.0 mg/dL
CA	H 9.7	5.9-9.4 mg/dL
NA	141	124-174 mmol/L
K	L 4.0	4.6-8.0 mmol/L
CL	112	92-120 mmol/L
CO2	19	mmol/L
AGAP	14.0	mmol/L
TP	4.7	3.6-6.6 g/dL
ALB	L 2.2	2.5-4.8 g/dL
GLOB	H 2.5	0.0-0.6 g/dL
A/G	0.9	
ALTv	29	28-132 U/L
ALKP	88	62-209 IU/L
GGT	<10	IU/L
TBIL	0.40	mg/dL
CHOL	88	36-96 mg/dL
AMY	2401	1691-3615 IU/L
LIPA	789	IU/L

Figure S46. Clinical blood report of the mice after 7 days of subcutaneous injection of 0.1 mL n-PBDF solution polymerized from 10mM BDF and 20µM Hb in PBS with 1wt% TPGS-750-M and 5% DMSO.

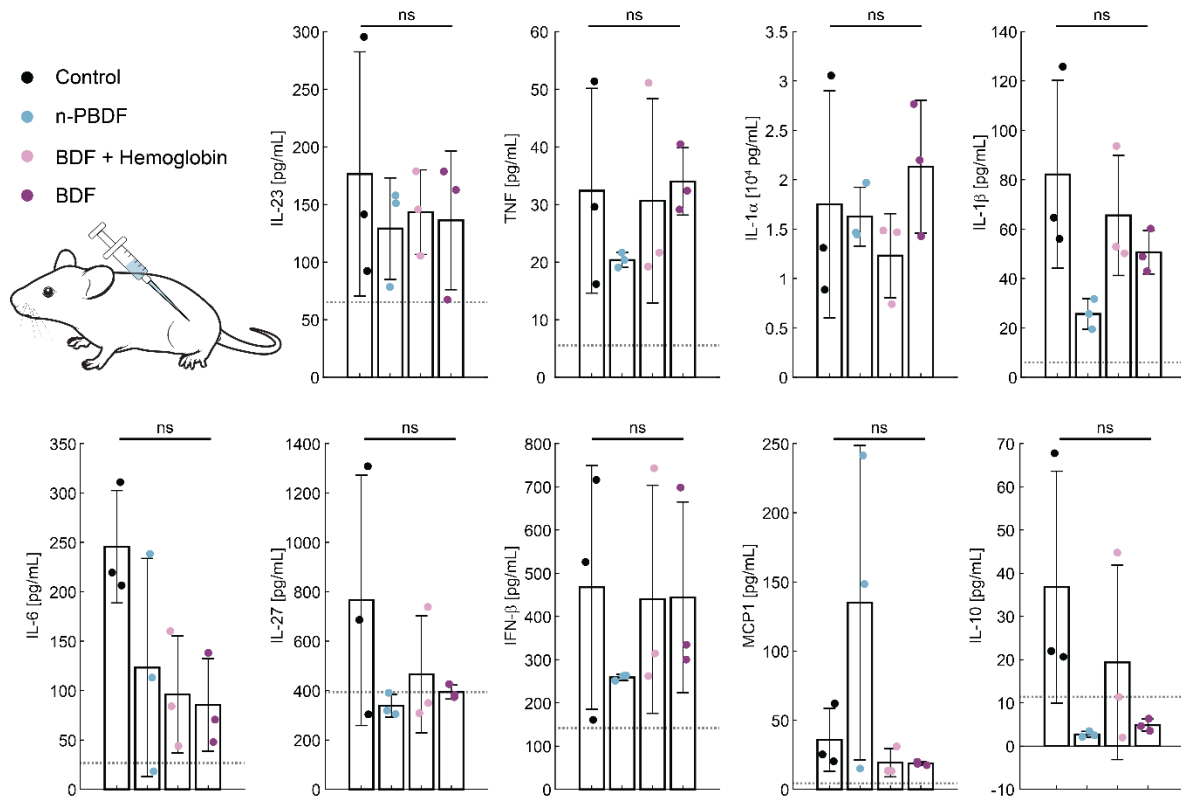


Figure S47. Inflammatory cytokine assay for mice with subcutaneous injections. Four groups of mice were injected with PBS (control), pre-formed n-PBDF (n-PBDF), BDF + hemoglobin injected after initiating the reaction for 30 minutes (BDF + Hemoglobin), and BDF, respectively. From top left to bottom right shows the concentration of the following Inflammatory cytokines in the skin tissue: IL-23, TNF, IL-1 α , IL-1 β , IL-6, IL-27, IFN- β , MCP1, and IL-10. The dotted line denotes the detection limits (n = 3 mice in each group, one-way ANOVA with post hoc Tukey-Kramer test).

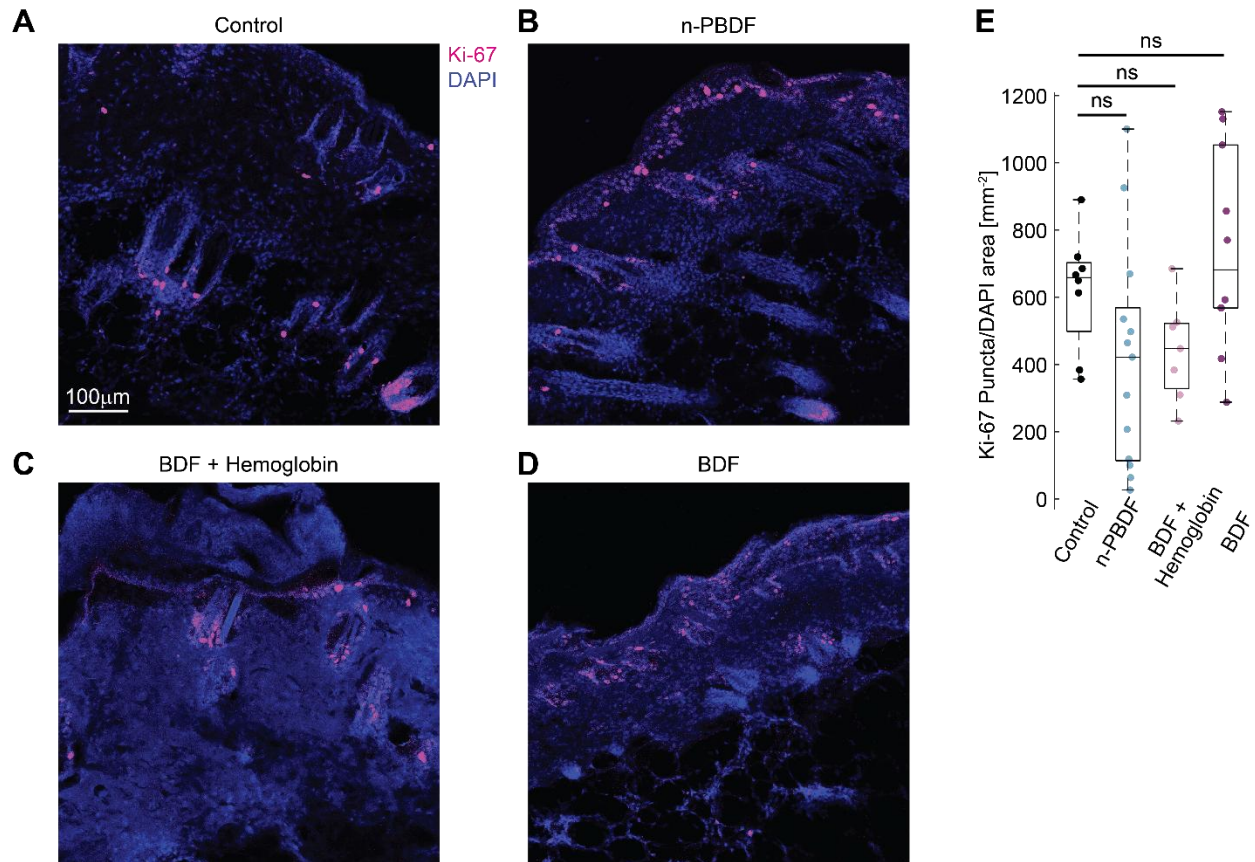


Figure S48. Ki-67 staining in subcutaneously injected skin tissues. Examples of skin tissues stained for Ki-67 from four groups of mice injected with (A) PBS, (B) pre-formed n-PBDF, (C) BDF + hemoglobin injected after initiating the reaction for 30 minutes, and (D) BDF monomer. (E) Number of Ki-67-positive cells normalized by DAPI-stained area from the four groups ($n = 3$ animals/group, one-way ANOVA with post hoc Tukey-Kramer test).

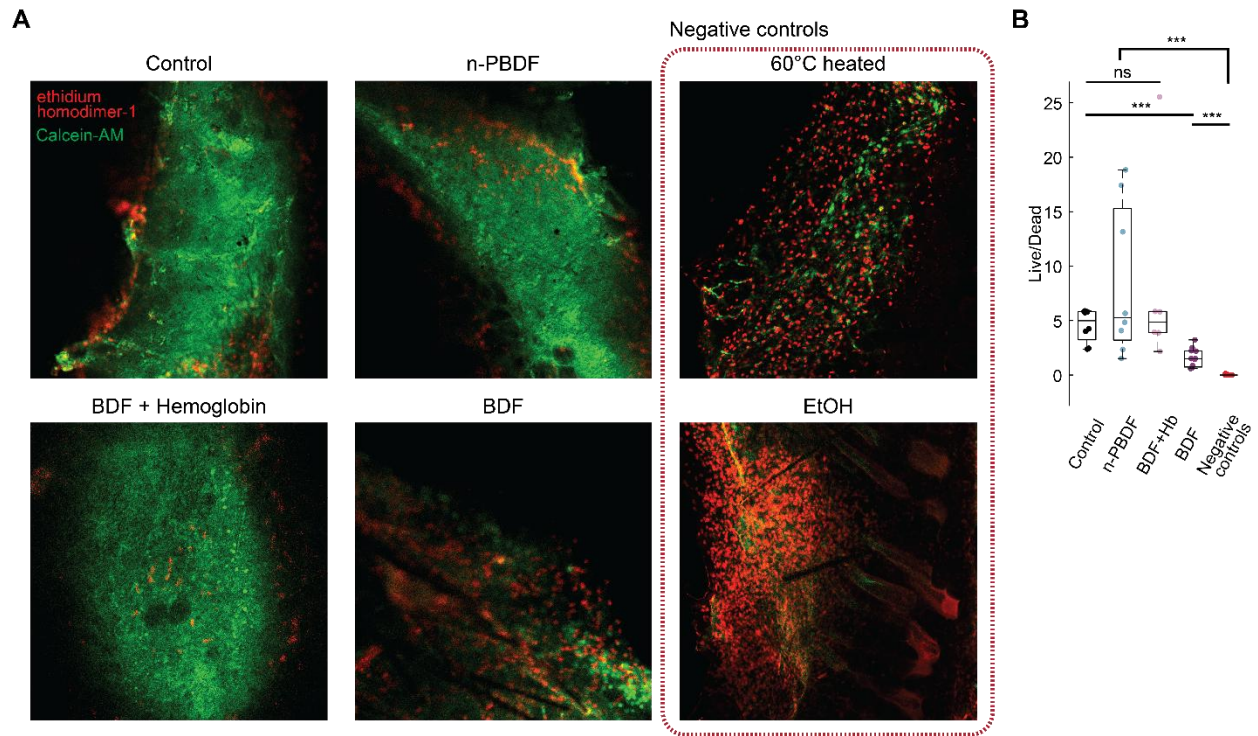


Figure S49. Cytotoxicity assay in subcutaneously injected skin tissues. **(A)** Examples of skin tissues stained with Calcein-AM and Ethidium homodimer-1 from four groups of mice injected with PBS (control), pre-formed n-PBDF (n-PBDF), BDF + hemoglobin injected after initiating the reaction for 30 minutes (BDF + Hemoglobin), and BDF monomer, respectively, and the negative control skin tissues treated with ethanol or heating. **(B)** The ratio between Calcein-AM positive area and Ethidium homodimer-1 positive area of the four treatment groups in comparison to the negative control groups (ethanol or heating treated) ($n = 2$ to 3 sections in each group, Wilcoxon-Mann-Whitney test).

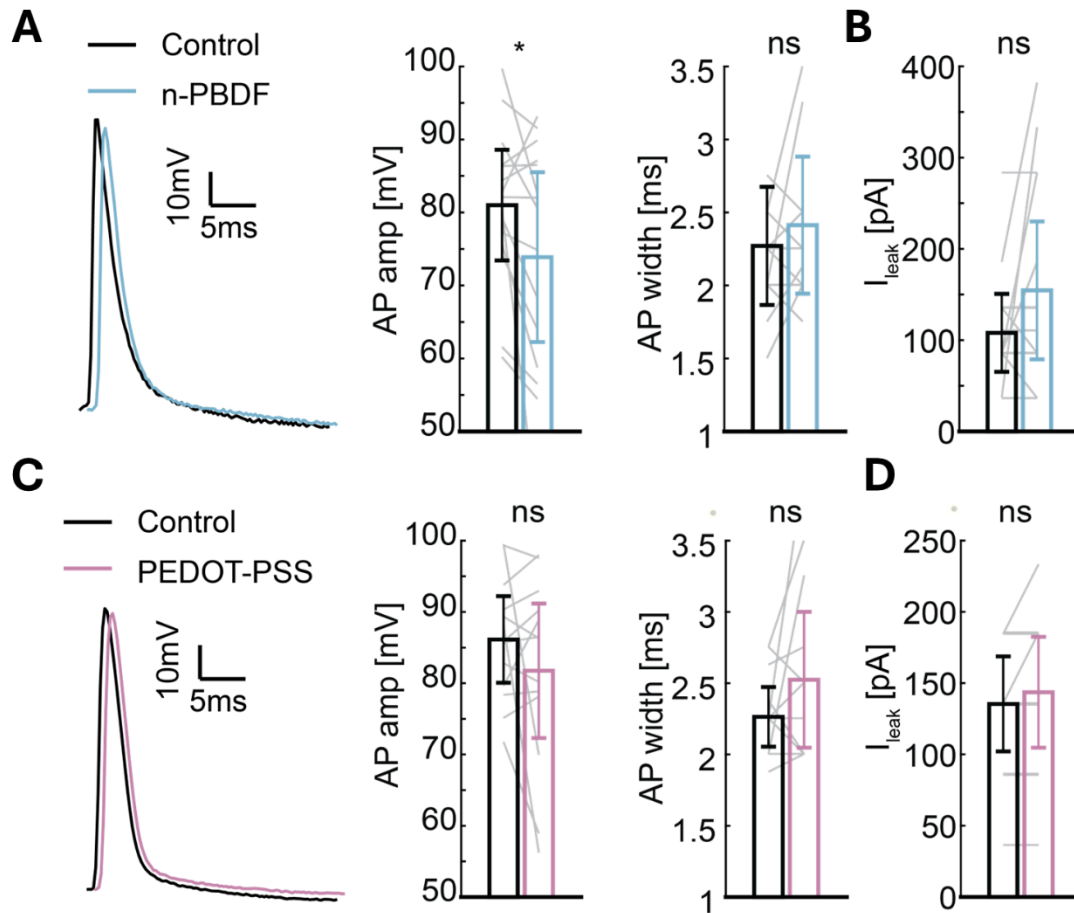


Figure S50. (A) n-PBDF slightly reduces the amplitude but doesn't change the width of action potentials (AP) ($n = 15$ neurons, Wilcoxon signed rank test, $*p < 0.05$), (B) n-PBDF doesn't change the leak current ($n = 17$ neurons, Wilcoxon signed rank test), (C) PEDOT: PSS doesn't change the amplitude or width of action potentials (AP) ($n = 13$ neurons, Wilcoxon signed rank test), and (D) PEDOT: PSS doesn't change the leak current ($n = 12$ neurons, Wilcoxon signed rank test), error bars: mean $\pm$ sem.

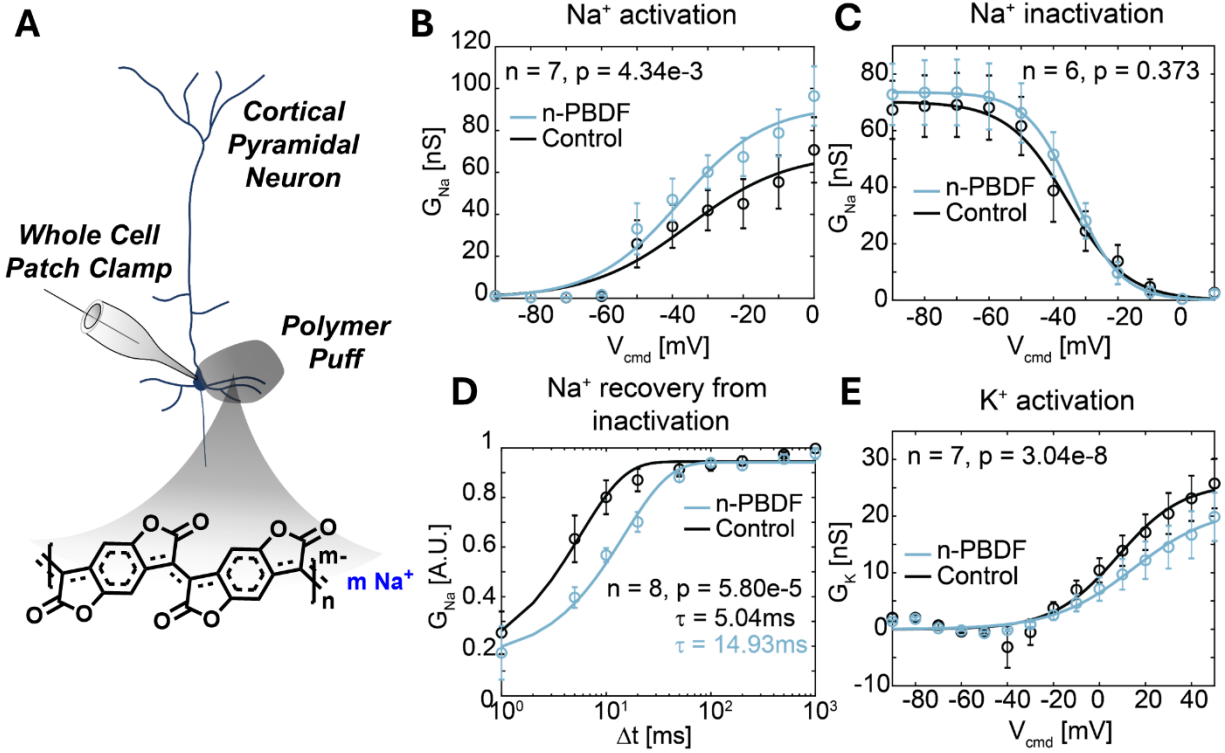


Figure S51. Impact of n-PBDF puff on the ion channels. **(A)** Schematic representation of n-PBDF local puff near a patch-clamped cortical pyramidal neuron in an acute brain slice, Voltage-dependent **(B)** activation ($n = 7$ neurons, repeated measures ANOVA, $p(10,60) < 0.001$) and **(C)** inactivation of Na^+ channels in pyramidal neurons before (black) and after n-PBDF puff (cyan) ($n = 6$ neurons, repeated measures ANOVA, $p(10,50) < 0.38$), **(D)** Na^+ channel recovery from inactivation before (black) and after n-PBDF puff (cyan) ($n = 8$ neurons, repeated measures ANOVA, $p(8,56) < 0.001$), and **(E)** Voltage-dependent activation of K^+ channels in pyramidal neurons before (black) and after n-PBDF puff (cyan) ($n = 7$ neurons, repeated measures ANOVA, $p(14,84) < 0.001$), error bars: mean $\pm$ sem.

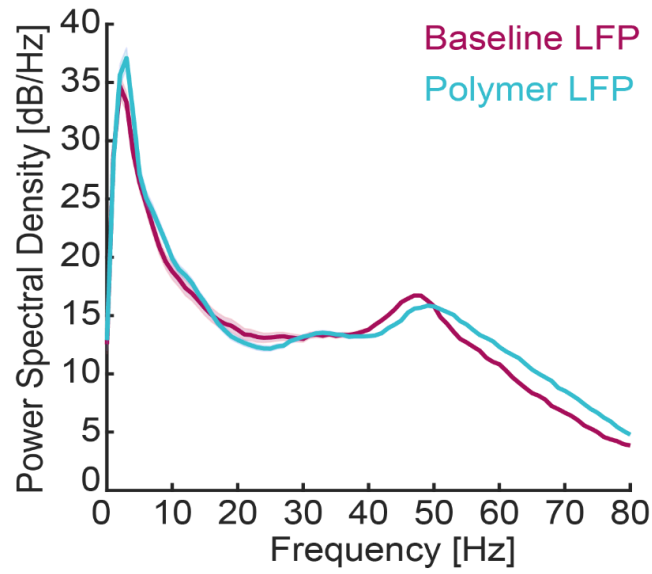
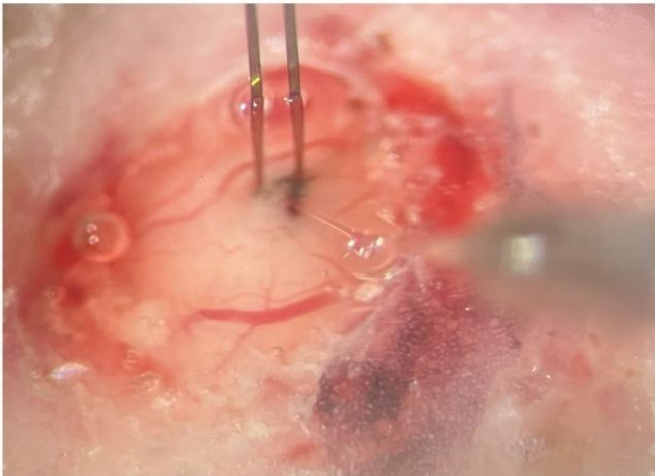


Figure S52. n-PBDF does not change the local field potential profile (representative plot from one of $n = 4$ mice).

A



B

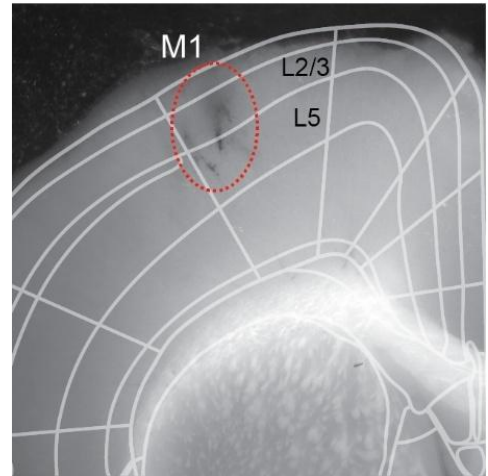


Figure S53. Spread of injected n-PBDF in the mouse brain during the *in vivo* recordings. **(A)** n-PBDF injected near the silicon probe during the *in vivo* recordings. **(B)** Spread of injected n-PBDF in the brain revealed in the brain slice (dissected 1 week after injection).

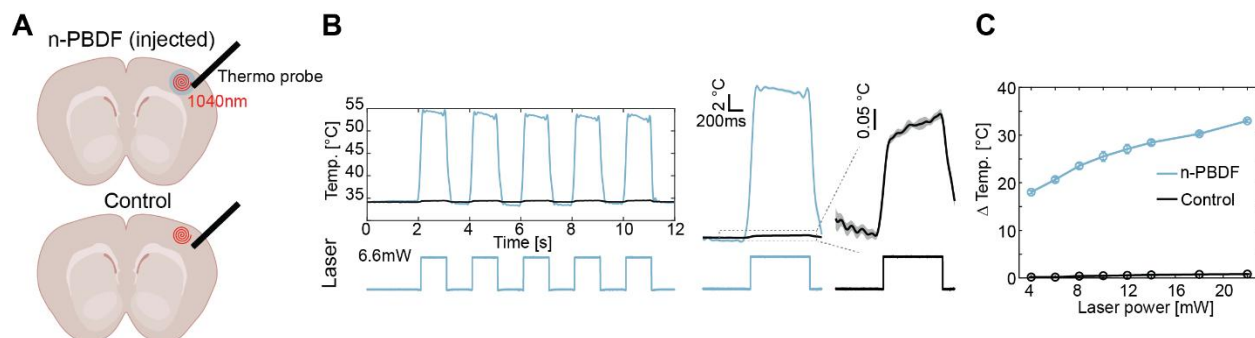


Figure S54. Photo-stimulation of n-PBDF changes the local temperature on tissue. (A) Schematic representation of detection of temperature change with and without the presence of n-PBDF on brain tissue using a thermal probe and NIR stimulation, (B) Temperature change with 1s NIR laser spiral pulse (1040 nm, 6.6mW) under control (black) and n-PBDF puff on the dendrite (cyan), and (C) Temperature change as a function of laser power under control (black) and n-PBDF puff on the brain tissue (cyan) ($n = 10$ measurements, repeated measures ANOVA, $p(1, 9) < 0.001$), error bars: mean $\pm$ sem.

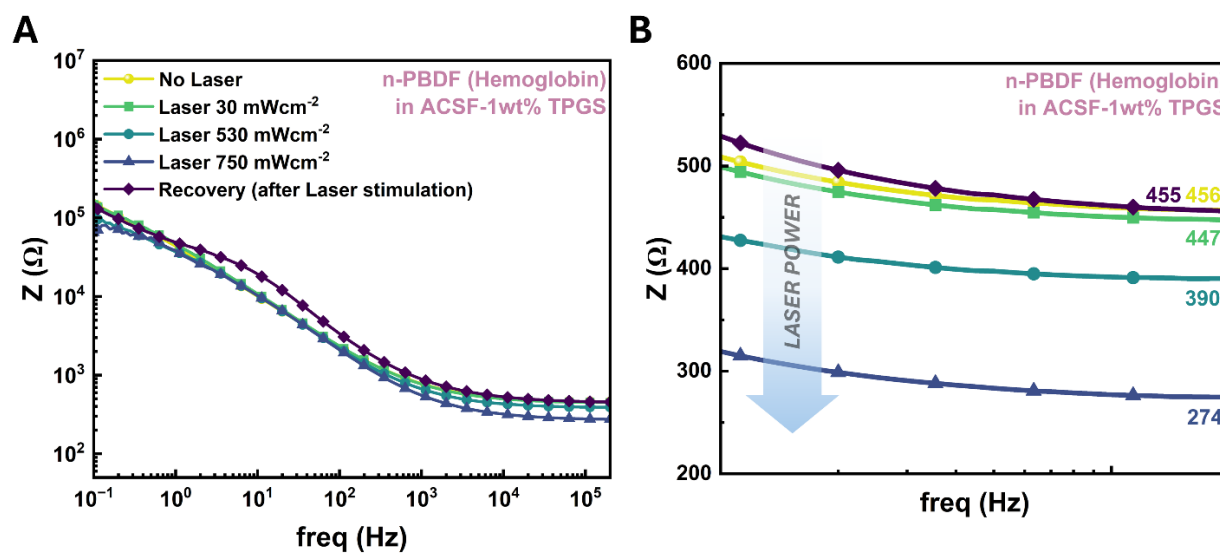


Figure S55. Photo-stimulation of n-PBDF changes the conductance of the polymer solution. (A) Bode plot of n-PBDF polymer solution (synthesized with Hb and without any purification) without and with laser stimulation at different laser power densities, and (B) Zoomed section of the high frequency range showing a decrease in polymer solution impedance as the laser power density is increased.

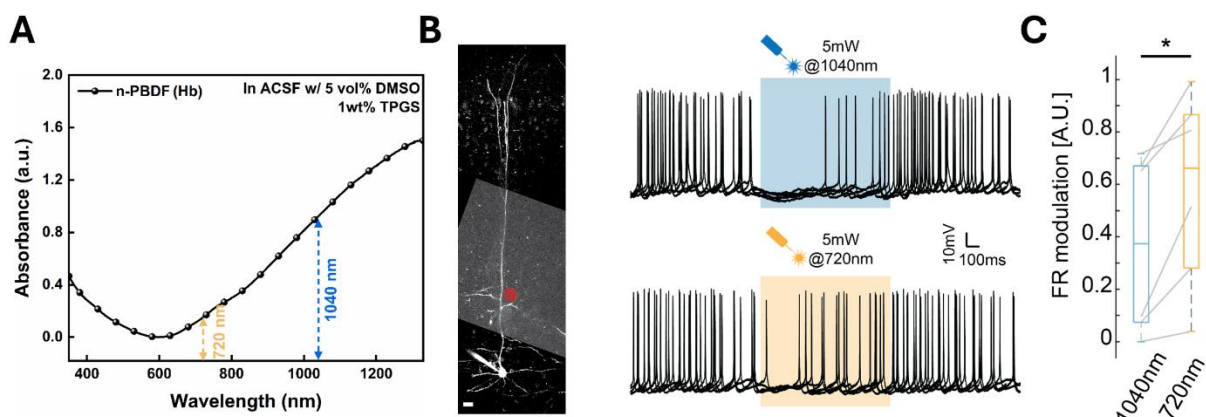


Figure S56. Photo-stimulation of n-PBDF using different wavelengths. (A) UV-Vis-NIR absorption profile of n-PBDF synthesized by hemoglobin in ACSF, (B) Photo-stimulation of n-PBDF near dendrites of L5 PN using NIR (1040nm) and red (720nm) lasers (scale bar: 20 μ m), and (C) 720nm stimulation induced less neuromodulation compared to 1040nm ($n = 6$, $p < 0.05$, Wilcoxon signed rank test).

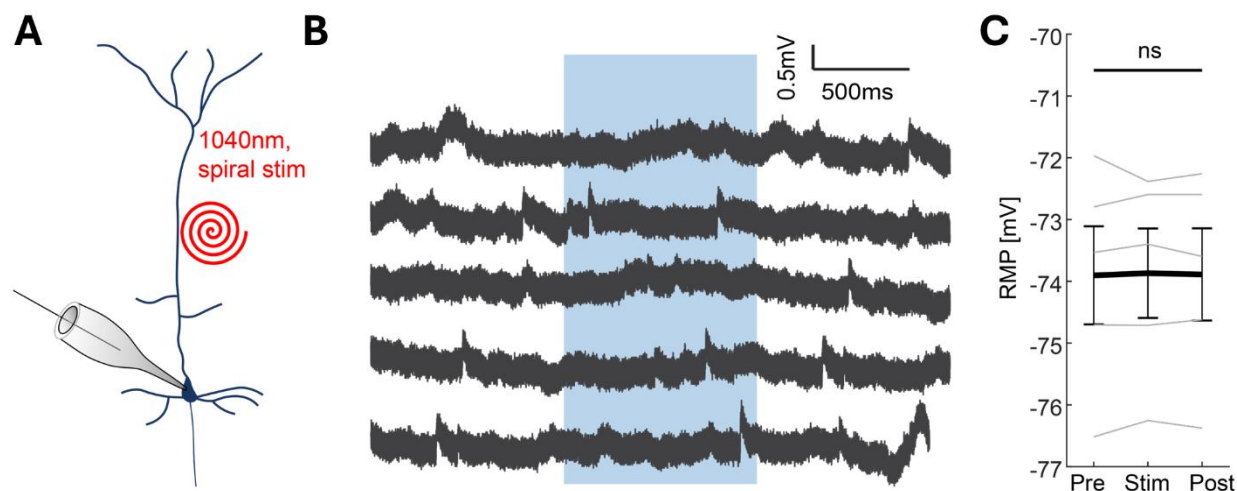


Figure S57. Two-photon NIR stimulation on dendrites doesn't change the RMP. (A) Schematic representation of NIR spiral stimulation on the dendrite, (B) NIR stimulation on dendrites without n-PBDF (1040nm, 6.6mW, 1s), and (C) Resting membrane potential (RMP) before, during, and after NIR stimulation ($n = 5$ neurons, paired sample t-test, $p > 0.7$), error bars: mean $\pm$ sem.

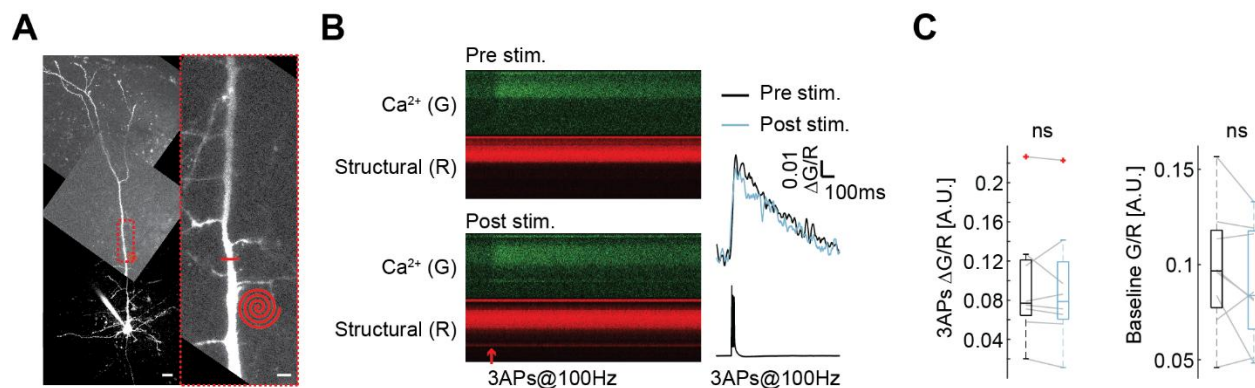


Figure S58. Dendritic Ca^{2+} imaging reveals minimum phototoxicity induced by photothermal modulation via n-PBDF. (A) Line scan performed on the dendritic segments near the photo-stimulation site, (B) Dendritic Ca^{2+} line scan performed before and after dendritic photo-stimulation, and (C) Photo-stimulation via n-PBDF doesn't change baseline or bAP-evoked dendritic Ca^{2+} levels ($n = 8$ neurons, Wilcoxon signed rank test).

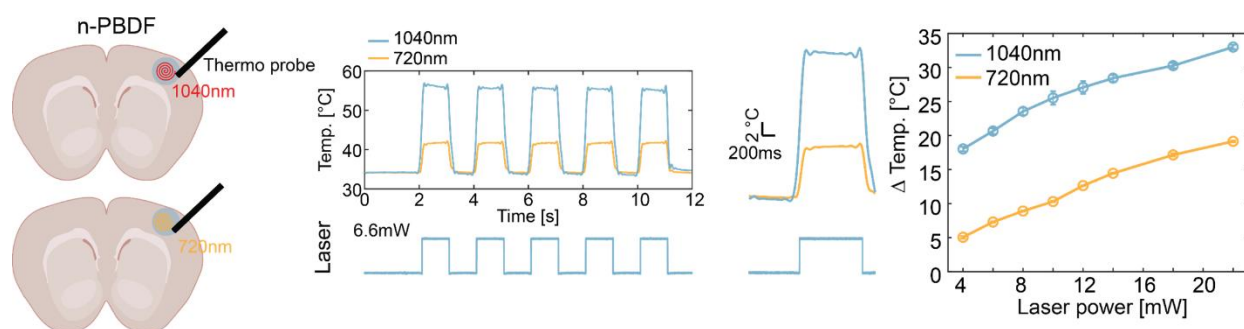


Figure S59. Temperature changes induced by photo-stimulation of n-PBDF using 720 nm and 1040 nm lasers ($n = 10$ measurements), error bars: mean $\pm$ sem.

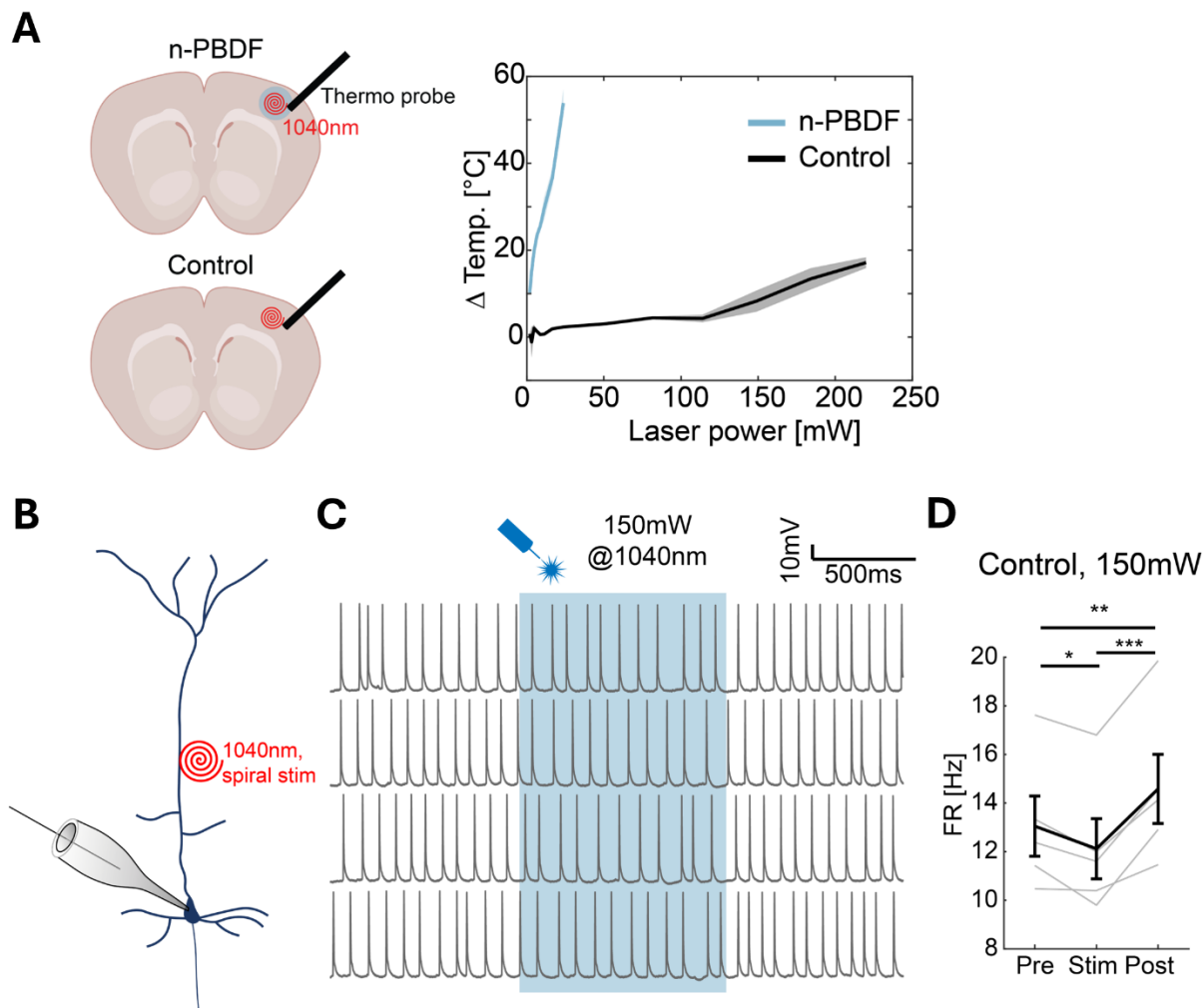


Figure S60. High power NIR stimulation on dendrites without n-PBDF causes light inhibition of spiking through heating. **(A)** Temperature change as a function of laser power under control (black) and n-PBDF puff on tissue (cyan) ($n = 10$ measurements), **(B)** Schematic representation of NIR spiral stimulation on the dendrite, **(C)** High power NIR stimulation on dendrites without n-PBDF (1040nm, 150mW, 1s), and **(D)** Firing rates measured before, during, and after the light stimulation in neurons showing slightly suppressed spiking but increased firing rate after light stimulation ($n = 5$ neurons, paired sample t-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$), error bars: mean $\pm$ sem.

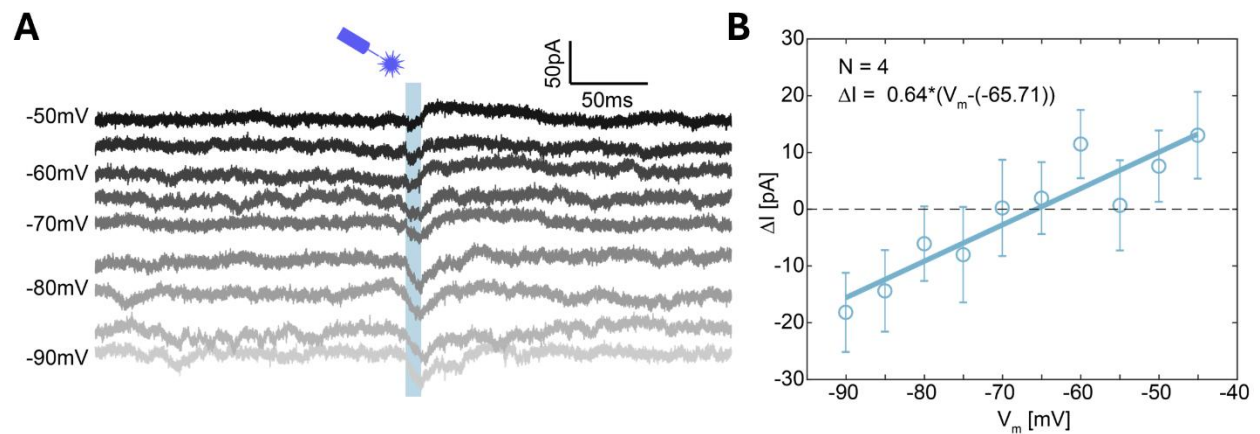


Figure S61. (A) Photo-evoked current measured using voltage clamp, and (B) The photo-evoked current revealed a reversal potential close to the resting membrane potential (RMP), error bars: mean $\pm$ sem.

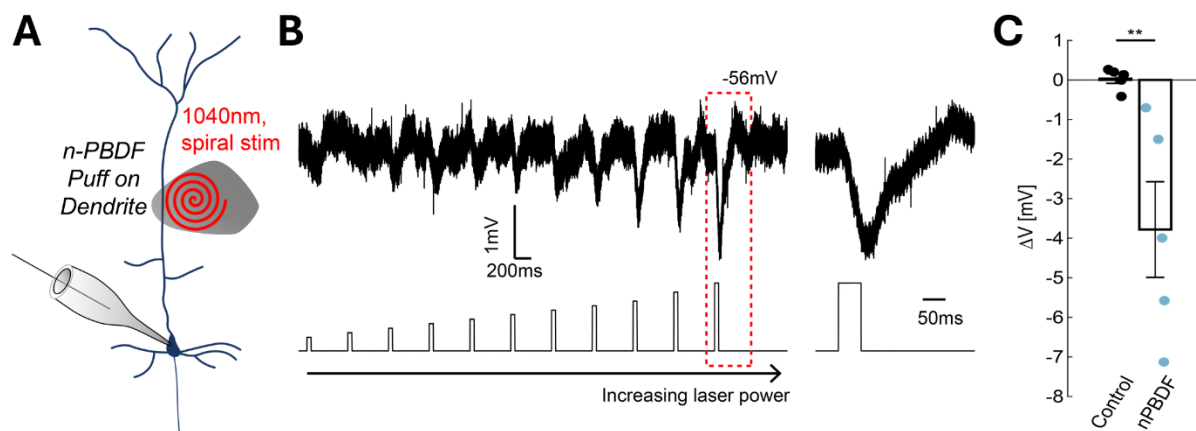


Figure S62. (A) Schematic representation of two-photon NIR stimulation on n-PBDF puffed on dendrites, (B) NIR light pulse-induced hyperpolarization with n-PBDF, and (C) Amplitude of light-induced hyperpolarization in control (black) and with n-PBDF (cyan) ($n = 5$ neurons for control, 5 for n-PBDF, two-sided Wilcoxon rank sum test, $p < 0.01$), error bars: mean $\pm$ sem.

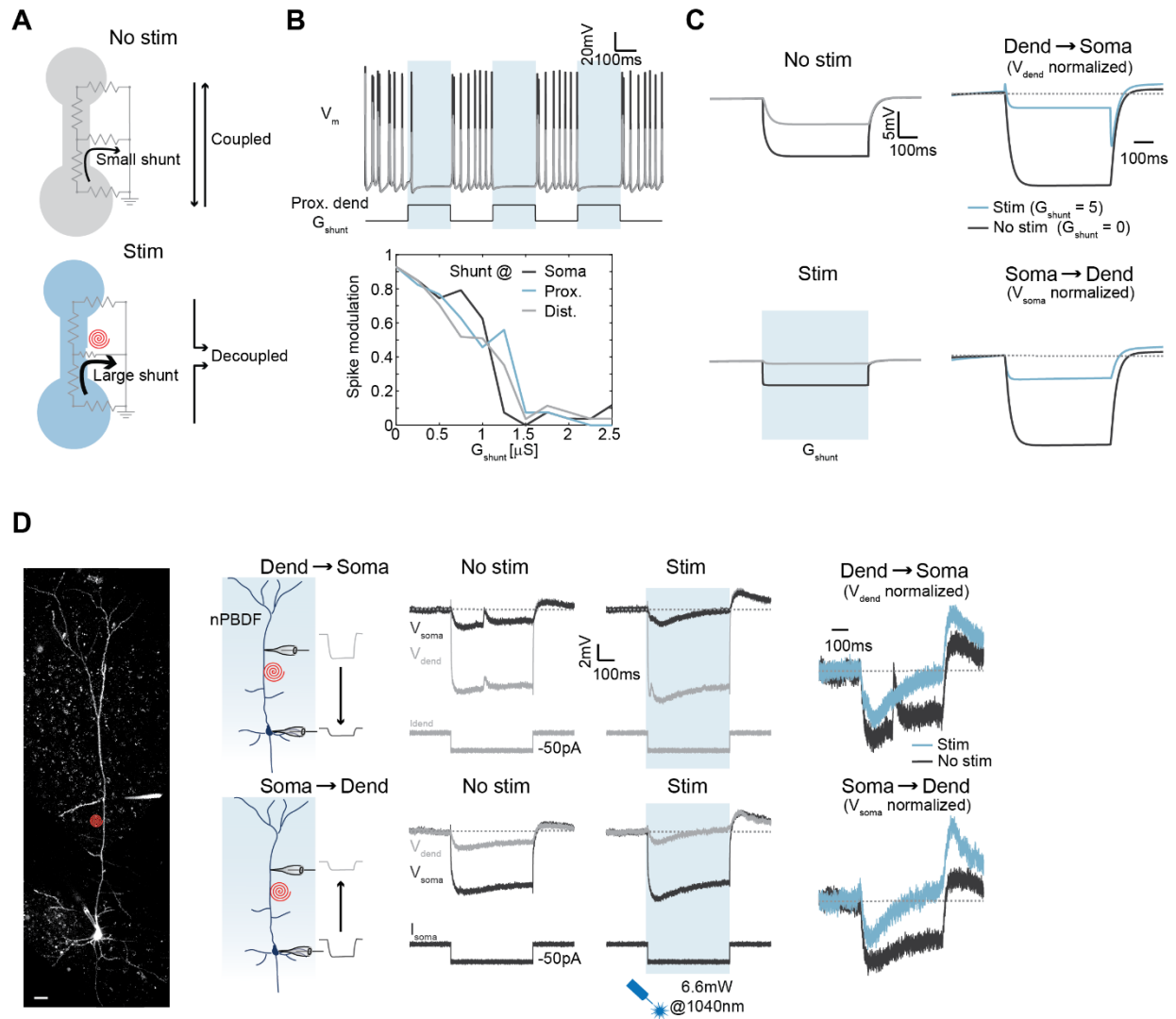


Figure S63. Photo-stimulation of n-PBDF induces dendritic shunting inhibition, which suppresses somatic firing. **(A)** A multicompartiment biophysical model for simulating the effect of dendritic shunting, **(B)** Shunting conductance on the proximal dendrite suppressed spiking in the simulation, **(C)** Shunting conductance on the proximal dendrite reduced coupling between the soma and the distal dendrite, and **(D)** Soma-dendritic dual patch during photo-stimulation of *in vivo* synthesized n-PBDF.

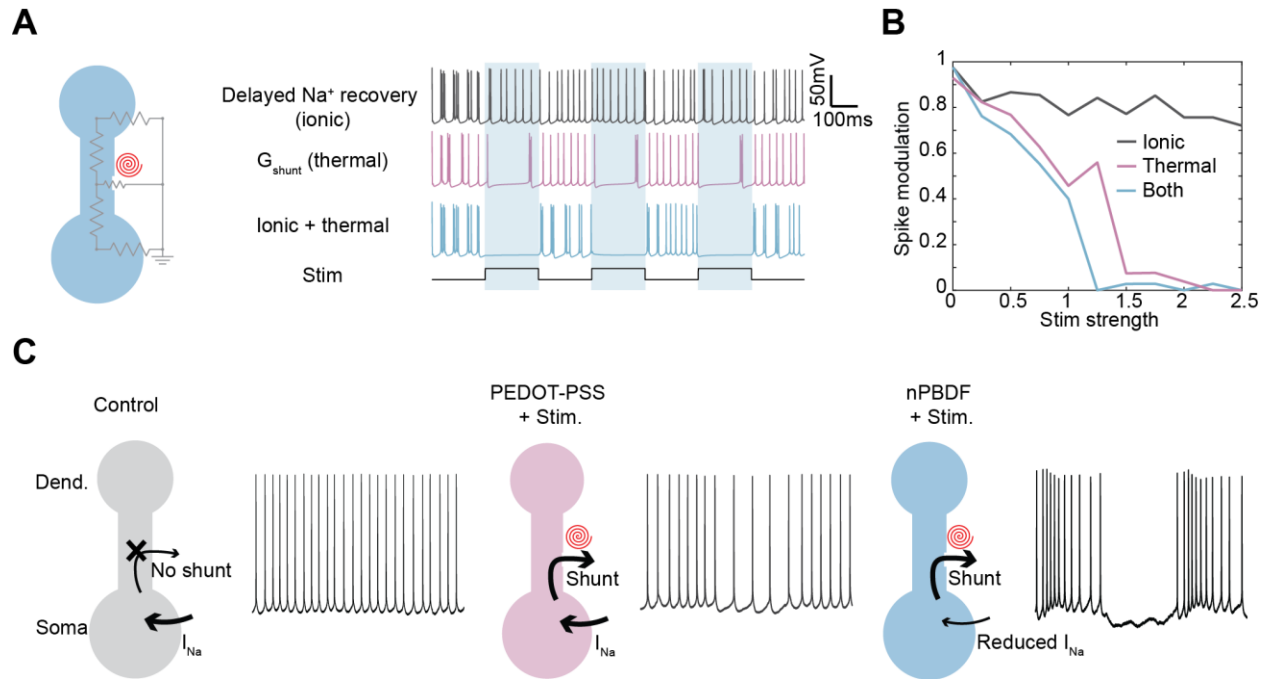


Figure S64. Combining ionic and thermal effects leads to more efficient optical neuromodulation. **(A)** Simulation reveals suppression of firing under different modulatory mechanisms applied to the dendrites, **(B)** A combination of both mechanisms leads to stronger suppression, and **(C)** Schematic representation of the mechanisms of photo-stimulation induced modulation by different conducting polymers: n-PBDF (cyan), and PEDOT: PSS (magenta).

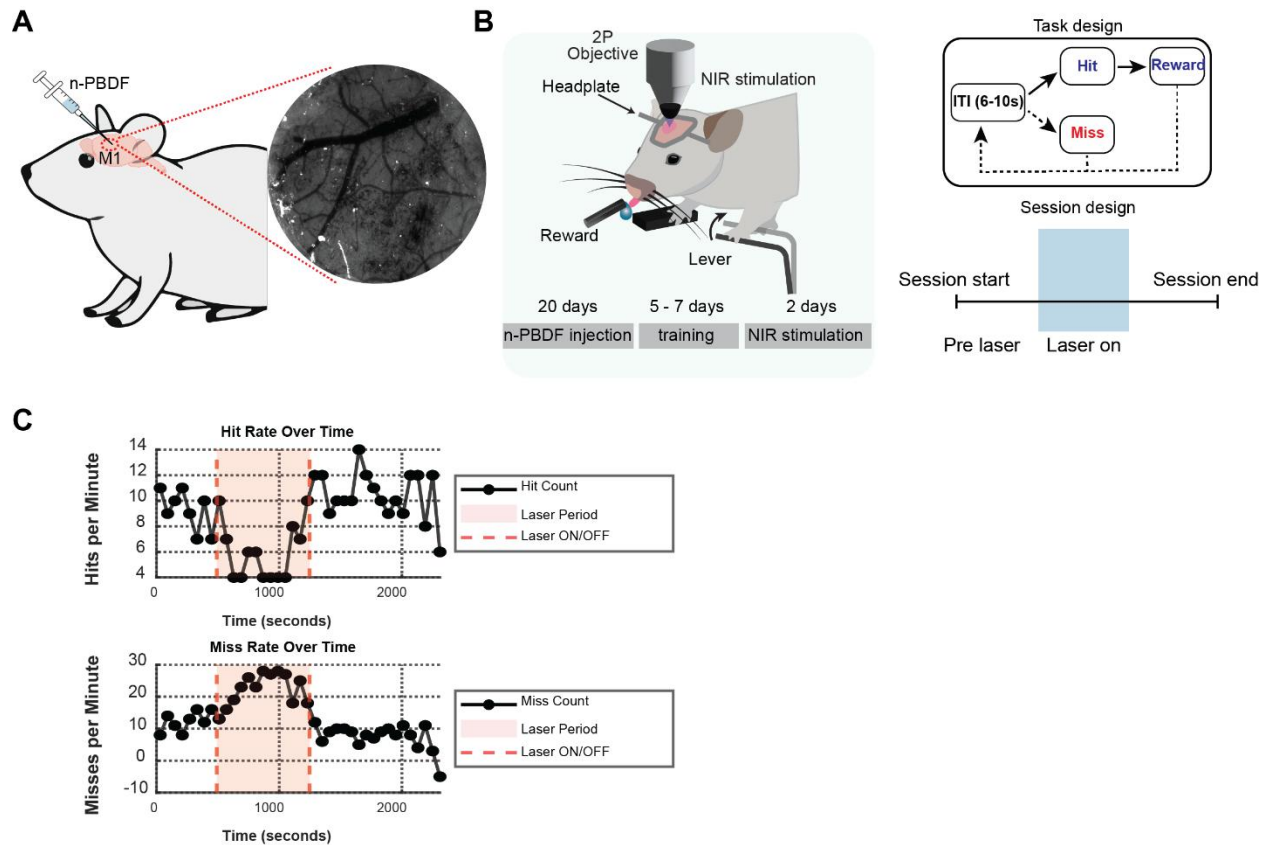


Figure S65. Photo-stimulation of n-PBDF injected *in vivo* alters lever-pulling performance. **(A)** Chronic cranial window with injected n-PBDF. **(B)** Schematics and timeline of the lever-pulling task, and **(C)** Photo-stimulation reliably alters the performance.

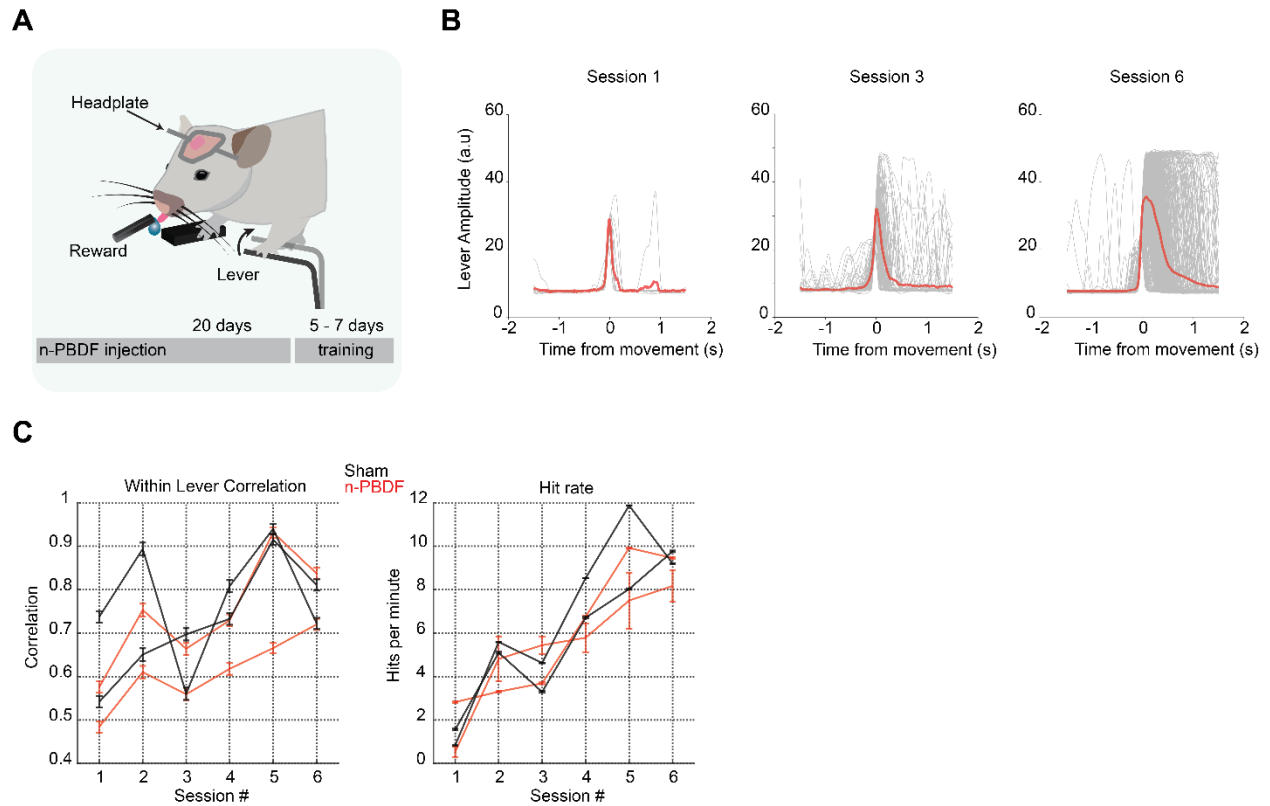


Figure S66. n-PBDF doesn't impact motor learning ability. **(A)** Schematics and timeline of mice injected with n-PBDF performing a motor learning task, **(B)** Examples showing the emergence of stereotypical lever trajectories as a function of learning, and **(C)** Similar motor learning ability observed in mice injected with n-PBDF compared to the control conditions.

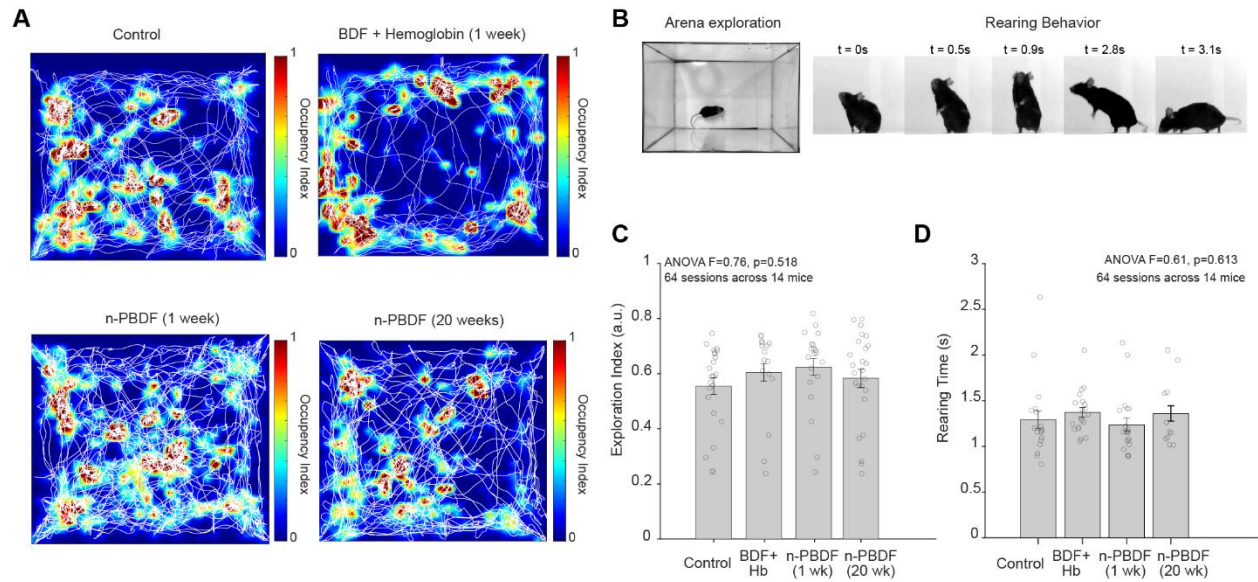


Figure S67. Mouse open-field exploration assay. **(A)** Open field test from 4 example mice that have been injected with artificial cerebral spinal fluid (ACSF), polymerization in vivo (BDF + hemoglobin injected after initiating the reaction for 30 minutes), and n-PBDF across a timeline spanning up to 20 weeks across groups. White traces corresponded to the position of the animal from which an occupancy map was derived to quantify exploration of the animal. **(B)** (Left) Top-down view of area exploration for an example mouse to characterize the exploration index. (Right) Time-lapse of an example rearing bout occurring during stationary exploration. **(C)** Exploration index quantified across control and treatment groups ($F_{\text{treatments}} = 0.76$, $p = 0.518$, two-way unbalanced Anova, 64 sessions across 14 mice, $n = 4, 6, 5$ mice for control, BDF + hemoglobin, and n-PBDF, respectively.) **(D)** Averaging rearing time across control and treatment groups ($F_{\text{treatments}} = 0.61$, $p = 0.61$, two-way unbalanced Anova, 64 sessions across 14 mice, $n = 4, 6, 5$ mice for control, BDF + hemoglobin, and n-PBDF, respectively.)

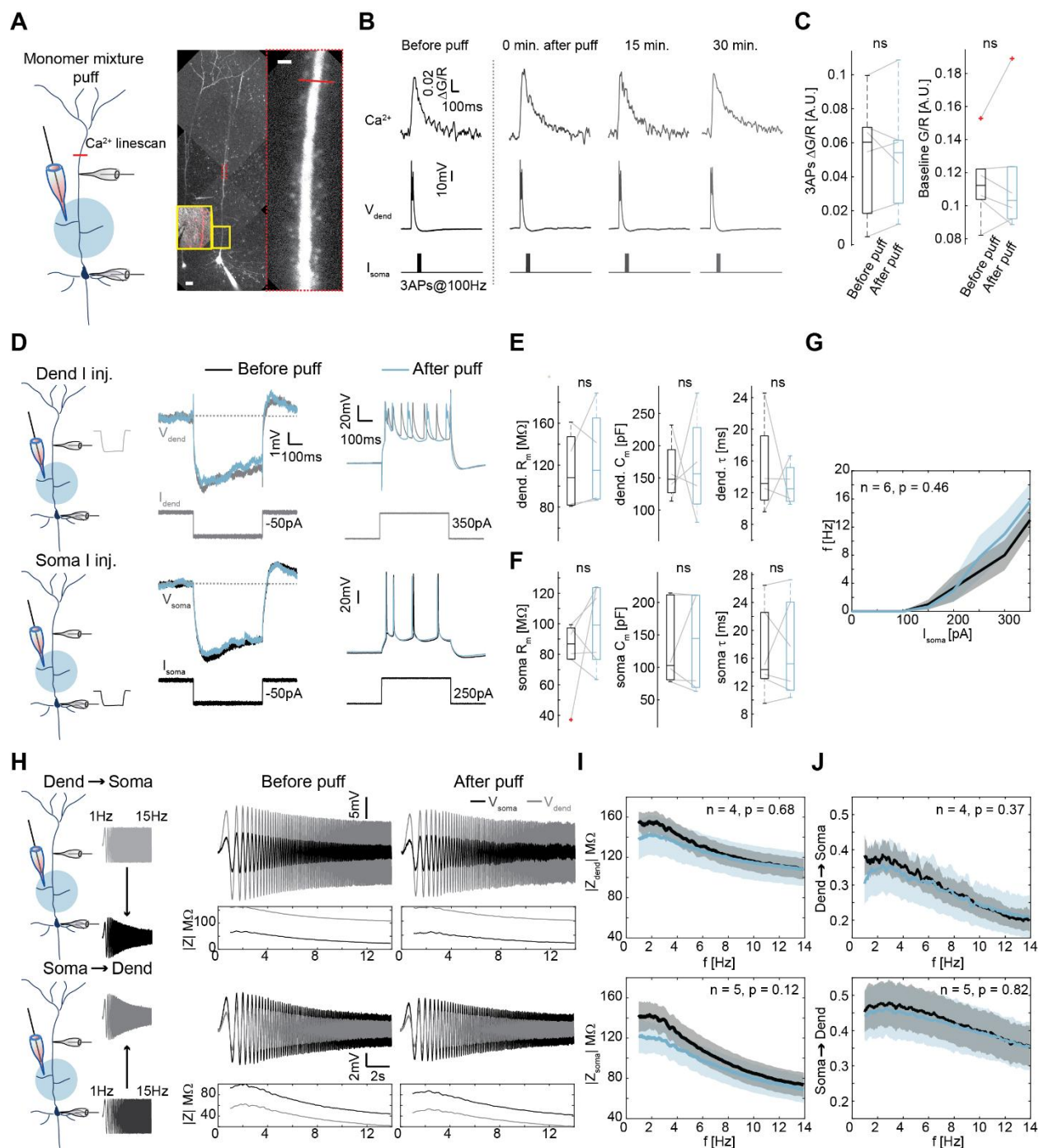


Figure S68. Minimum changes in Ca^{2+} signaling and soma and dendritic membrane electrical properties were induced by puff injection of the BDF mixture (BDF + hemoglobin after initiating the reaction for around 30 minutes). (A) Schematics of the soma-dendritic dual patch and Ca^{2+} imaging during puff injection of BDF and hemoglobin after 30 minutes of initializing the reaction. (B) Example dendritic back-propagated action potential (bAPs) and Ca^{2+} activities evoked by a somatic burst of action potentials (3 APs at 100Hz) before and after puff injection. (C) No changes

in the baseline and bAP-evoked dendritic Ca^{2+} signaling were induced by the puff of monomer mixture ($n = 6$ neurons, Wilcoxon signed rank test). **(D)** Example showing the forward (top) and backward (bottom) propagation of membrane voltage before and after the puff injection **(E)** No changes in the dendritic membrane resistance, membrane capacitance and time constant induced by the puff injection ($n = 4$ neurons, Wilcoxon signed rank test). **(F)** Similar to E, but for somatic membrane properties ($n = 6$ neurons, Wilcoxon signed rank test). **(G)** No changes in neuronal excitability ($n = 6$, repeated measures ANOVA). **(H)** Example showing the membrane impedance (measured from 1 to 15Hz) of soma and dendrite before and after the puff injection. **(I)** No changes in the dendritic (top) and somatic (bottom) impedance ($n = 4$ neurons for dendrite, $n = 5$ for soma, repeated measures ANOVA). **(J)** No changes in the forward and backward dendritic filtering properties induced by the puff injection ($n = 4$ neurons for dendrite, $n = 5$ for soma, repeated measures ANOVA).

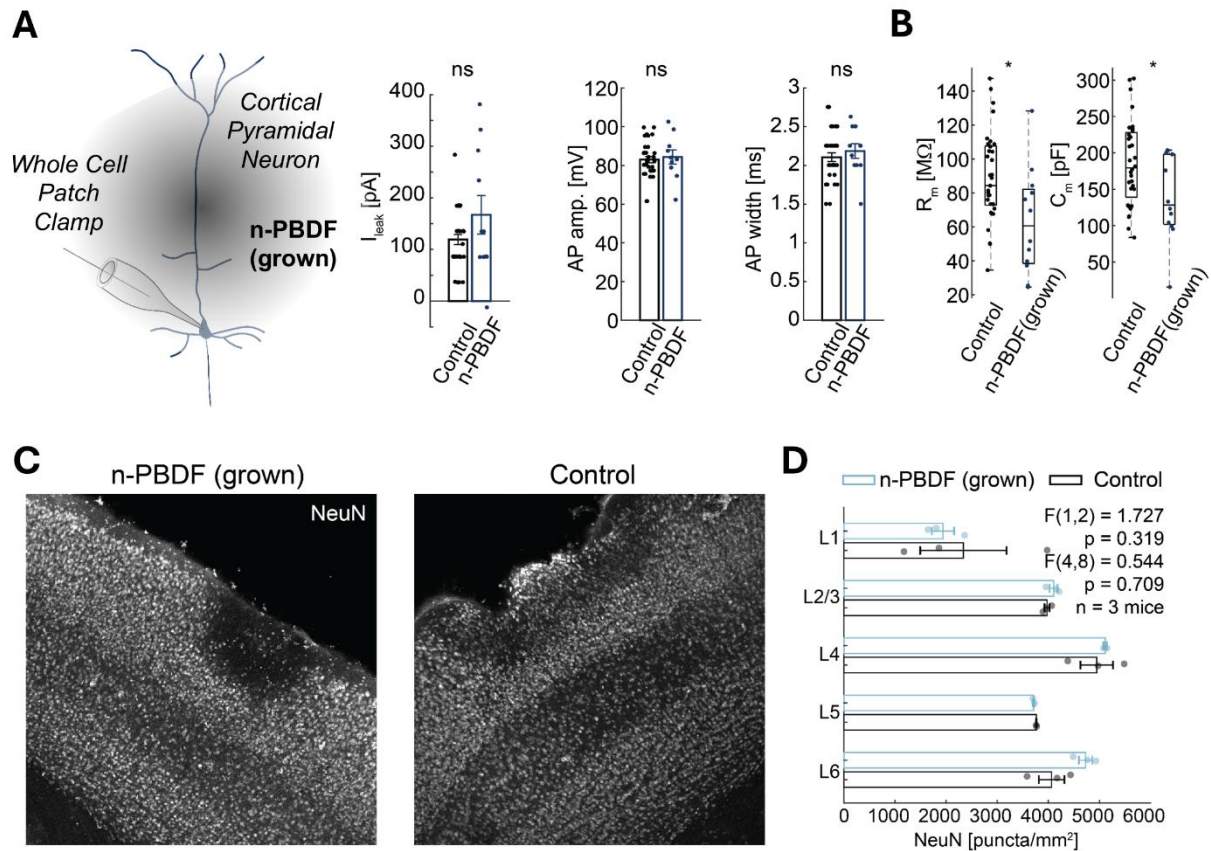


Figure S69. Minimum cytotoxicity induced by *in vivo* polymerized (grown) n-PBDF. **(A)** *In vivo* polymerization doesn't change the leak current or the action potential shape of neighboring neurons ($n = 33$ control, 12 n-PBDF, $p > 0.5$, Wilcoxon rank sum test), **(B)** Membrane resistance and capacitance of neurons neighboring *in vivo* synthesized n-PBDF compared to those under control conditions ($n = 33$ control, 12 n-PBDF, $*p < 0.05$, Wilcoxon rank sum test), **(C)** Examples of nucleus staining one week after *in vivo* polymerization or injection of control buffers, and **(D)** *In vivo* polymerization doesn't reduce the number of neurons compared to the injection of control buffers, error bars: mean $\pm$ sem.

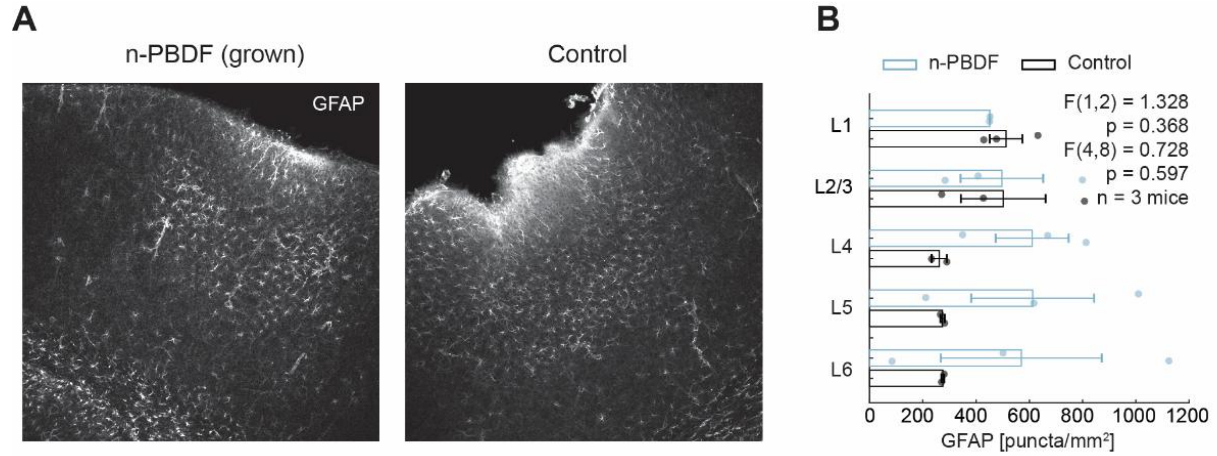


Figure S70. The gliosis induced by *in vivo* polymerization of n-PBDF is negligible. **(A)** An example of glia staining one week after *in vivo* polymerization or injection of control buffers, and **(B)** *In vivo* polymerization doesn't induce gliosis compared to the injection of control buffer, error bars: mean $\pm$ sem.

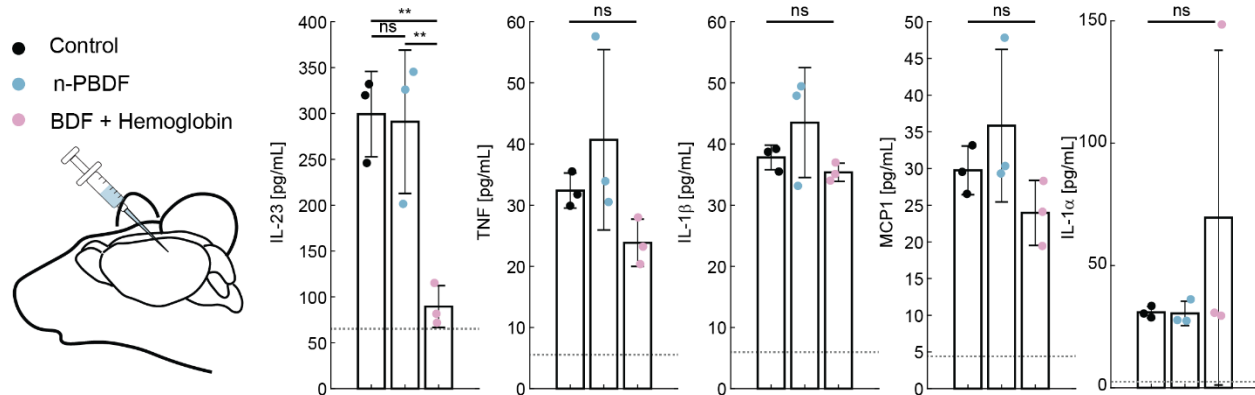


Figure S71. Inflammatory cytokine assay for mice with intracranial injections. Three groups of mice were injected with ACSF (control), pre-formed n-PBDF (n-PBDF), and BDF + hemoglobin injected after initiating the reaction for 30 minutes (BDF + Hemoglobin), respectively. From left to right shows the concentration of the following Inflammatory cytokines in the brains: IL-23, TNF, IL-1 β , MCP1, and IL-1 α . The dotted line denotes the detection limits ($n = 3$ mice in each group, one-way ANOVA with post hoc Tukey-Kramer test, ** $p < 0.01$).

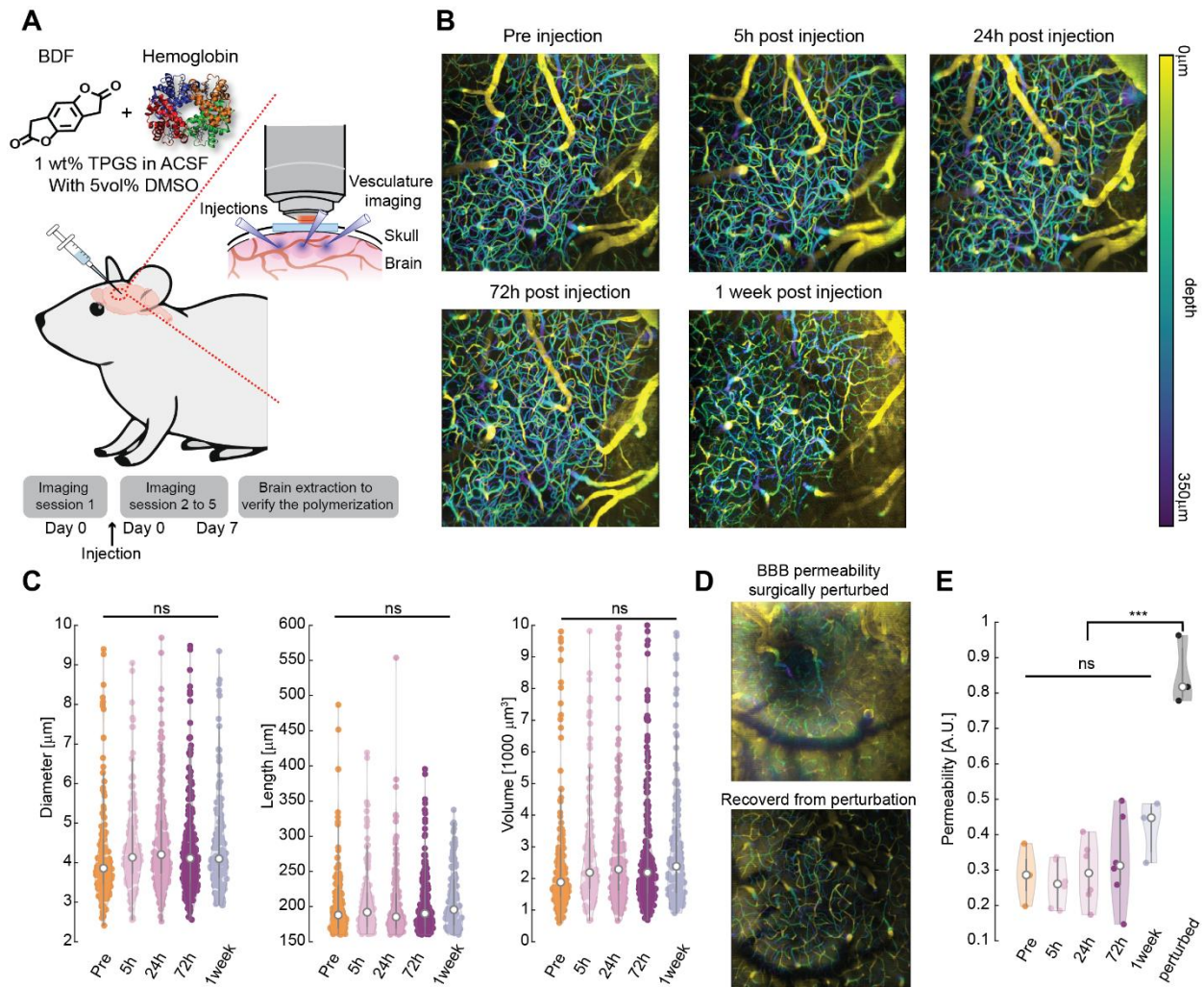


Figure S72. Chronic *in vivo* blood vessel imaging revealed minimum vessel dilation or compromised blood-brain-barrier (BBB) permeability caused by *in vivo* polymerization. **(A)** Schematics and timeline of the blood vessel imaging. **(B)** The vasculature images from before injection until one week post-injection. **(C)** No change in the diameter, length, and volume of blood vessels across the imaging sessions caused by the *in vivo* polymerization (n = around 200 vessels from 8 FOVs across two mice, ANOVA with nested design). **(D)** Example showing the elevated background fluorescence with perturbed BBB permeability. **(E)** BBB permeability remained intact by the *in vivo* polymerization (n = 8 FOVs across two mice, one-way ANOVA with post hoc Tukey-Kramer test).

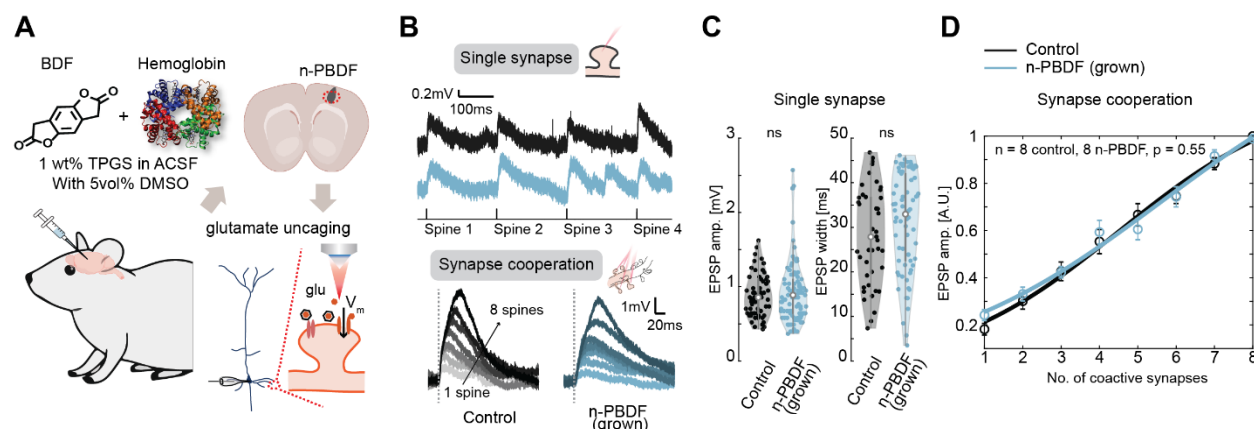


Figure S73. Two-photon glutamate uncaging revealed minimum extrasynaptic glutamate signaling induced by *in vivo* polymerization. **(A)** Schematics of the glutamate uncaging at the *in vivo* polymerization site. **(B)** (Top) The excitatory postsynaptic potentials (EPSPs) evoked by glutamate uncaging on 4 example spines on a neuron at the *in vivo* polymerization site (cyan, n-PBDF (grown)) and far from the injection site (black, control). (Bottom) The EPSPs evoked by gradually increasing the number of coactivated neighboring dendritic spines. **(C)** No changes in the amplitude, an halfwidth of unitary EPSPs caused by the *in vivo* polymerization ($n = 64$ dendritic spines for control and 104 dendritic spines for n-PBDF, Wilcoxon–Mann–Whitney test, $p > 0.1$), and **(D)** Example showing the elevated background fluorescence with perturbed BBB permeability ($n = 8$ dendritic branches for control and 8 for n-PBDF, two-way ANOVA, $p > 0.5$).

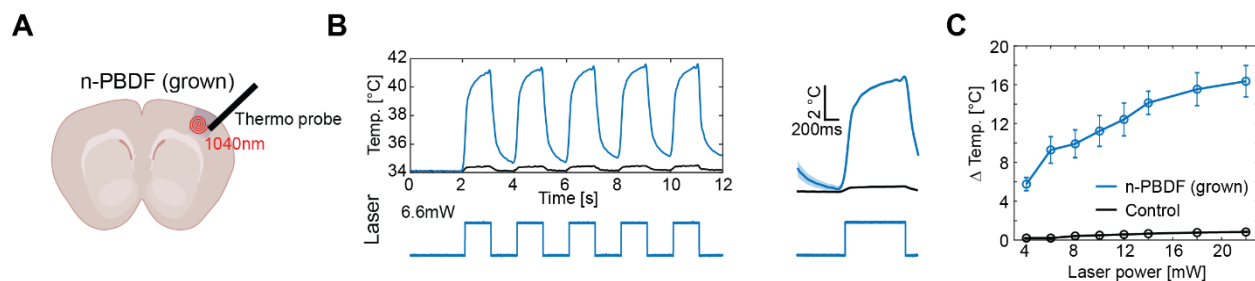


Figure S74. Photo-stimulation of *in vivo* synthesized n-PBDF changes the local temperature on tissue. (A) Schematic representation of detection of temperature change with the presence of *in vivo* synthesized n-PBDF on brain tissue using a thermal probe and NIR stimulation, control conditions were taken in neighboring brain regions without n-PBDF, (B) Temperature changes with 1s NIR laser spiral pulse (1040nm, 6.6mW) under control (black) and *in vivo* synthesized n-PBDF (blue), and (C) Temperature change as a function of laser power under control (black) and *in vivo* synthesized n-PBDF (blue) ($n = 10$ measurements, repeated measures ANOVA, $p(1,9) < 0.001$), error bars: mean $\pm$ sem.

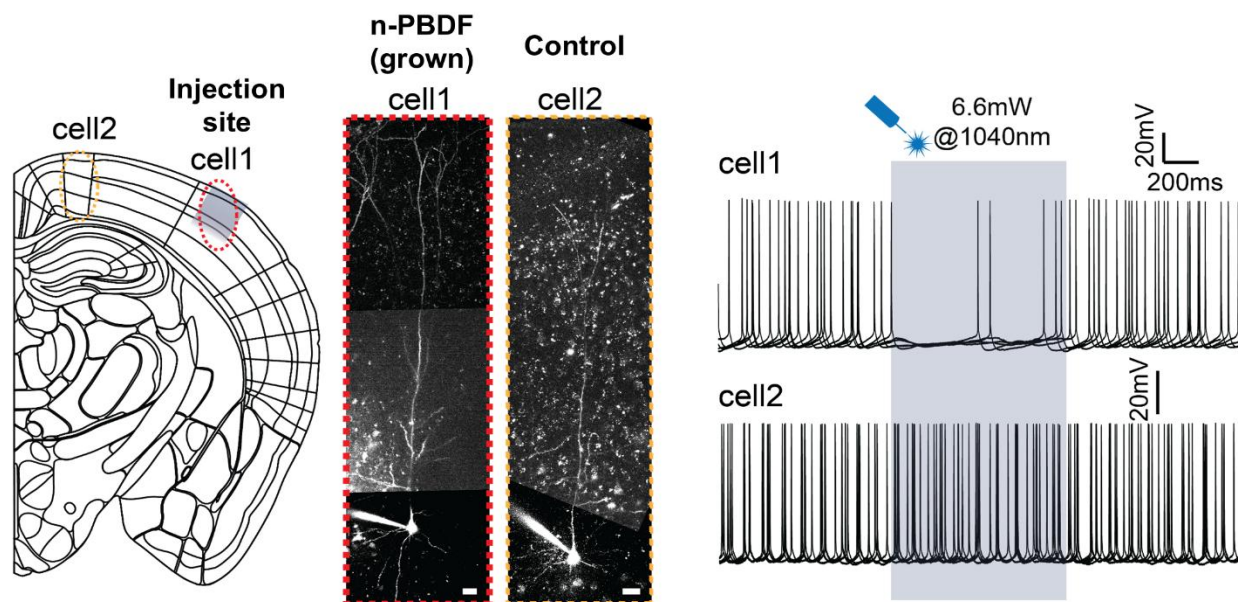


Figure S75. Spread of *in vivo* polymerized n-PBDF: Minimal photothermal neuromodulation away from the injected brain region. (Scale bar: 20µm)

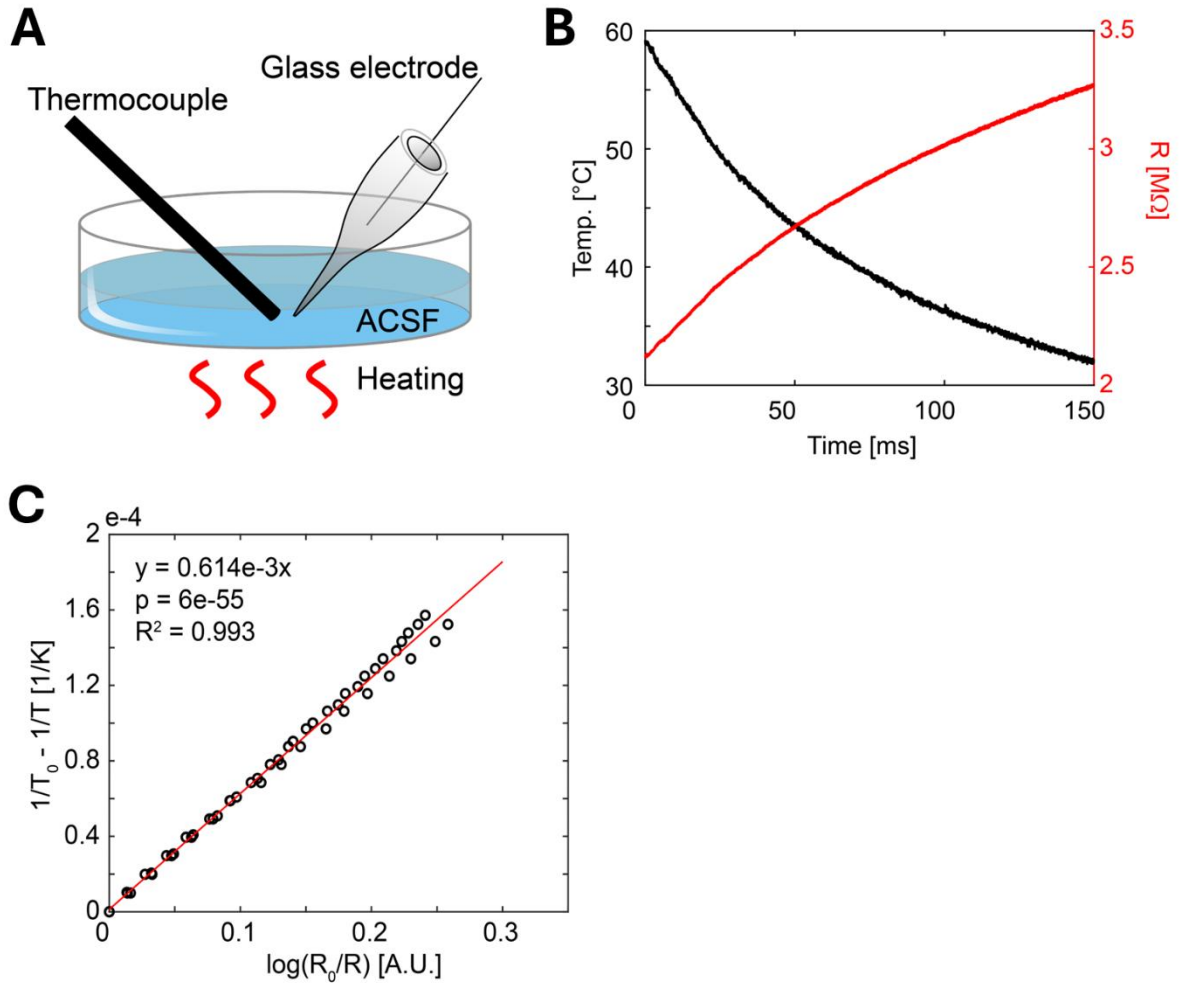


Figure S76. Calibration of thermos measurements using glass electrodes. **(A)** Schematic representation of the temperature measurement calibration, **(B)** An example of temperature (black, measured with a thermocouple) and glass pipette resistance (red) changing as the heated ACSF cools down, and **(C)** The relationship between pipette resistance and ACSF bath temperature.

References and notes

1. M. Berggren, E. D. Głowacki, D. T. Simon, E. Stavrinidou, K. Tybrandt, *In Vivo Organic Bioelectronics for Neuromodulation*. *Chem. Rev.* **122**, 4826–4846 (2022). [doi:10.1021/acs.chemrev.1c00390](https://doi.org/10.1021/acs.chemrev.1c00390) [Medline](#)
2. O. Bettucci, G. M. Matrone, F. Santoro, Conductive Polymer-Based Bioelectronic Platforms toward Sustainable and Biointegrated Devices: A Journey from Skin to Brain across Human Body Interfaces. *Adv. Mater. Technol.* **7**, 2100293 (2022). [doi:10.1002/admt.202100293](https://doi.org/10.1002/admt.202100293)
3. H. Lin, H. Bai, Z. Yang, Q. Shen, M. Li, Y. Huang, F. Lv, S. Wang, Conjugated polymers for biomedical applications. *Chem. Commun.* **58**, 7232–7244 (2022). [doi:10.1039/D2CC02177C](https://doi.org/10.1039/D2CC02177C) [Medline](#)
4. V. Guarino, S. Zuppolini, A. Borriello, L. Ambrosio, Electro-Active Polymers (EAPs): A Promising Route to Design Bio-Organic/Bioinspired Platforms with on Demand Functionalities. *Polymers* **8**, 185 (2016). [doi:10.3390/polym8050185](https://doi.org/10.3390/polym8050185) [Medline](#)
5. I. B. Dimov, M. Moser, G. G. Malliaras, I. McCulloch, Semiconducting Polymers for Neural Applications. *Chem. Rev.* **122**, 4356–4396 (2022). [doi:10.1021/acs.chemrev.1c00685](https://doi.org/10.1021/acs.chemrev.1c00685) [Medline](#)
6. T. Nezakati, A. Seifalian, A. Tan, A. M. Seifalian, Conductive Polymers: Opportunities and Challenges in Biomedical Applications. *Chem. Rev.* **118**, 6766–6843 (2018). [doi:10.1021/acs.chemrev.6b00275](https://doi.org/10.1021/acs.chemrev.6b00275) [Medline](#)
7. L. Benny Mattam, A. Bijoy, D. Abraham Thadathil, L. George, A. Varghese, Conducting Polymers: A Versatile Material for Biomedical Applications. *ChemistrySelect* **7**, e202201765 (2022). [doi:10.1002/slct.202201765](https://doi.org/10.1002/slct.202201765)
8. S. Inal, Turning tissues into conducting matter. *Science* **379**, 758–759 (2023). [doi:10.1126/science.adg4761](https://doi.org/10.1126/science.adg4761) [Medline](#)
9. A. Zhang, S. Zhao, J. Tyson, K. Deisseroth, Z. Bao, Applications of synthetic polymers directed toward living cells. *Nat. Synth.* **3**, 943–957 (2024). [doi:10.1038/s44160-024-00560-2](https://doi.org/10.1038/s44160-024-00560-2) [Medline](#)
10. J. Liu, Y. S. Kim, C. E. Richardson, A. Tom, C. Ramakrishnan, F. Birey, T. Katsumata, S. Chen, C. Wang, X. Wang, L. M. Joubert, Y. Jiang, H. Wang, L. E. Fenno, J. B. H. Tok, S. P. Paşca, K. Shen, Z. Bao, K. Deisseroth, Genetically targeted chemical assembly of functional materials in living cells, tissues, and animals. *Science* **367**, 1372–1376 (2020). [doi:10.1126/science.aay4866](https://doi.org/10.1126/science.aay4866) [Medline](#)
11. C. D. Sessler, Y. Zhou, W. Wang, N. D. Hartley, Z. Fu, D. Graykowski, M. Sheng, X. Wang, J. Liu, Optogenetic polymerization and assembly of electrically functional polymers for modulation of single-neuron excitability. *Sci. Adv.* **8**, eade1136 (2022). [doi:10.1126/sciadv.ade1136](https://doi.org/10.1126/sciadv.ade1136) [Medline](#)
12. A. Zhang, K. Y. Loh, C. S. Kadur, L. Michalek, J. Dou, C. Ramakrishnan, Z. Bao, K. Deisseroth, Genetically targeted chemical assembly of polymers specifically localized extracellularly to surface membranes of living neurons. *Sci. Adv.* **9**, eadi1870 (2023).

13. A. Zhang, Y. Jiang, K. Y. Loh, Z. Bao, K. Deisseroth, Genetically targeted chemical assembly. *Nat. Rev. Bioeng.* **2**, 82–94 (2024). [doi:10.1038/s44222-023-00110-z](https://doi.org/10.1038/s44222-023-00110-z)
14. X. Strakosas, H. Biesmans, T. Abrahamsson, K. Hellman, M. S. Ejneby, M. J. Donahue, P. Ekström, F. Ek, M. Savvakis, M. Hjort, D. Bliman, M. Linares, C. Lindholm, E. Stavriniidou, J. Y. Gerasimov, D. T. Simon, R. Olsson, M. Berggren, Metabolite-induced in vivo fabrication of substrate-free organic bioelectronics. *Science* **379**, 795–802 (2023). [doi:10.1126/science.adc9998](https://doi.org/10.1126/science.adc9998) [Medline](#)
15. G. Tommasini, M. De Simone, S. Santillo, G. Dufil, M. Iencharelli, D. Mantione, E. Stavriniidou, A. Tino, C. Tortiglione, In vivo neuromodulation of animal behavior with organic semiconducting oligomers. *Sci. Adv.* **9**, eadi5488 (2023). [doi:10.1126/sciadv.adi5488](https://doi.org/10.1126/sciadv.adi5488) [Medline](#)
16. Y. Wang, R. Garg, D. Cohen-Karni, T. Cohen-Karni, Neural modulation with photothermally active nanomaterials. *Nat. Rev. Bioeng.* **1**, 193–207 (2023). [doi:10.1038/s44222-023-00022-y](https://doi.org/10.1038/s44222-023-00022-y) [Medline](#)
17. W. Wang, C. D. Sessler, X. Wang, J. Liu, In Situ Synthesis and Assembly of Functional Materials and Devices in Living Systems. *Acc. Chem. Res.* **57**, 2013–2026 (2024). [doi:10.1021/acs.accounts.4c00049](https://doi.org/10.1021/acs.accounts.4c00049) [Medline](#)
18. S. Jiang, X. Wu, N. J. Rommelfanger, Z. Ou, G. Hong, Shedding light on neurons: Optical approaches for neuromodulation. *Natl. Sci. Rev.* **9**, nwac007 (2022). [doi:10.1093/nsr/nwac007](https://doi.org/10.1093/nsr/nwac007) [Medline](#)
19. H. Tang, Y. Liang, C. Liu, Z. Hu, Y. Deng, H. Guo, Z. Yu, A. Song, H. Zhao, D. Zhao, Y. Zhang, X. Guo, J. Pei, Y. Ma, Y. Cao, F. Huang, A solution-processed n-type conducting polymer with ultrahigh conductivity. *Nature* **611**, 271–277 (2022). [doi:10.1038/s41586-022-05295-8](https://doi.org/10.1038/s41586-022-05295-8) [Medline](#)
20. Z. Ke, A. Abtahi, J. Hwang, K. Chen, J. Chaudhary, I. Song, K. Perera, L. You, K. N. Baustert, K. R. Graham, J. Mei, Highly Conductive and Solution-Processable n-Doped Transparent Organic Conductor. *J. Am. Chem. Soc.* **145**, 3706–3715 (2023). [doi:10.1021/jacs.2c13051](https://doi.org/10.1021/jacs.2c13051) [Medline](#)
21. G. Liu, H. H. Hsu, S. Samal, W. J. Lee, Z. Ke, L. You, B. M. Savoie, J. Mei, Selenium Dioxide Catalyzed Polymerization of N-doped Poly(benzodifurandione) (n-PBDF) and Its Derivatives. *Angew. Chem. Int. Ed.* **64**, e202418668 (2025). [doi:10.1002/anie.202418668](https://doi.org/10.1002/anie.202418668) [Medline](#)
22. Q. Li, J.-D. Huang, T. Liu, T. P. A. van der Pol, Q. Zhang, S. Y. Jeong, M.-A. Stoeckel, H.-Y. Wu, S. Zhang, X. Liu, H. Y. Woo, M. Fahlman, C.-Y. Yang, S. Fabiano, A Highly Conductive n-Type Conjugated Polymer Synthesized in Water. *J. Am. Chem. Soc.* **146**, 15860–15868 (2024). [doi:10.1021/jacs.4c02270](https://doi.org/10.1021/jacs.4c02270) [Medline](#)
23. G. Liu, U. Pal, S. Samal, M. F. Espenship, Y. Li, W. J. Lee, L. A. Ogunfowora, L. You, J. Laskin, J. Mei, Catalytic Polymerization of n-Doped Poly(benzodifurandione) (n-PBDF) Using Parts Per Million (ppm) Levels of Molybdenum Trioxide. *Angew. Chem. Int. Ed.* **64**, e202510411 (2025). [doi:10.1002/anie.202510411](https://doi.org/10.1002/anie.202510411) [Medline](#)
24. H. Cai, L. Shao, X. Duan, S. Li, X. Qin, C. Liu, H. Tang, Y. Cao, F. Huang, Solvent-promoted synthesis of n-type high-conductivity poly(benzodifurandione) (PBFDO) via

- DMSO/Ac₂O: An efficient, purification-free approach. *Sci. China Chem.* **69**, 819–825 (2025). [doi:10.1007/s11426-025-2814-5](https://doi.org/10.1007/s11426-025-2814-5)
25. B. H. Lipshutz, S. Ghorai, A. R. Abela, R. Moser, T. Nishikata, C. Duplais, A. Krasovskiy, R. D. Gaston, R. C. Gadwood, TPGS-750-M: A second-generation amphiphile for metal-catalyzed cross-couplings in water at room temperature. *J. Org. Chem.* **76**, 4379–4391 (2011). [doi:10.1021/jo101974u](https://doi.org/10.1021/jo101974u) [Medline](#)
26. E. El Maaiden, Y. El Kharrassi, A. K. Essamadi, K. Moustaid, B. Nasser, Improved Method for DNA Extraction and Purification from *Tetrahymena pyriformis*. *Methods Protoc.* **2**, 40 (2019). [doi:10.3390/mps2020040](https://doi.org/10.3390/mps2020040) [Medline](#)
27. R. Piña, A. I. Santos-Díaz, E. Orta-Salazar, A. R. Aguilar-Vazquez, C. A. Mantellero, I. Acosta-Galeana, A. Estrada-Mondragon, M. Prior-Gonzalez, J. I. Martinez-Cruz, A. Rosas-Arellano, Ten Approaches That Improve Immunostaining: A Review of the Latest Advances for the Optimization of Immunofluorescence. *Int. J. Mol. Sci.* **23**, 1426 (2022). [doi:10.3390/ijms23031426](https://doi.org/10.3390/ijms23031426) [Medline](#)
28. H. Sun, R. Yang, J. Wang, X. Yang, J. Tu, L. Xie, C. Li, Q. Lao, C. Sun, Component-based biocompatibility and safety evaluation of polysorbate 80. *RSC Adv.* **7**, 15127–15138 (2017). [doi:10.1039/C6RA27242H](https://doi.org/10.1039/C6RA27242H)
29. S. Enthaler, K. Junge, M. Beller, Sustainable metal catalysis with iron: From rust to a rising star? *Angew. Chem. Int. Ed.* **47**, 3317–3321 (2008). [doi:10.1002/anie.200800012](https://doi.org/10.1002/anie.200800012) [Medline](#)
30. G. Pishchany, E. P. Skaar, Taste for blood: Hemoglobin as a nutrient source for pathogens. *PLOS Pathog.* **8**, e1002535 (2012). [doi:10.1371/journal.ppat.1002535](https://doi.org/10.1371/journal.ppat.1002535) [Medline](#)
31. J. D. Morris, D. Khanal, J. A. Richey, C. K. Payne, Hemoglobin-mediated synthesis of PEDOT:PSS: enhancing conductivity through biological oxidants. *Biomater. Sci.* **3**, 442–445 (2015). [doi:10.1039/C4BM00338A](https://doi.org/10.1039/C4BM00338A) [Medline](#)
32. J. J. Flores, C. K. Payne, J. D. Morris, Heme protein-mediated synthesis of PEDOT:PSS: enhancing conductivity by inhibiting heme degradation. *RSC Adv.* **7**, 12017–12021 (2017). [doi:10.1039/C7RA00887B](https://doi.org/10.1039/C7RA00887B)
33. D. Ohayon, G. Quek, B. R. P. Yip, F. Lopez-Garcia, P. R. Ng, R. J. Vázquez, D. V. Andreeva, X. Wang, G. C. Bazan, High-Performance Aqueous Supercapacitors Based on a Self-Doped n-Type Conducting Polymer. *Adv. Mater.* **36**, e2410512 (2024). [doi:10.1002/adma.202410512](https://doi.org/10.1002/adma.202410512) [Medline](#)
34. R. Dong, P. X. Ma, B. Guo, Conductive biomaterials for muscle tissue engineering. *Biomaterials* **229**, 119584 (2020). [doi:10.1016/j.biomaterials.2019.119584](https://doi.org/10.1016/j.biomaterials.2019.119584) [Medline](#)
35. J. H. Lee, Y. C. Yoon, H. S. Kim, J. Lee, E. Kim, C. Findelee, U. Katscher, In vivo electrical conductivity measurement of muscle, cartilage, and peripheral nerve around knee joint using MR-electrical properties tomography. *Sci. Rep.* **12**, 73 (2022). [doi:10.1038/s41598-021-03928-y](https://doi.org/10.1038/s41598-021-03928-y) [Medline](#)
36. G. Kougkolos, M. Golzio, L. Laudebat, Z. Valdez-Nava, E. Flahaut, Hydrogels with electrically conductive nanomaterials for biomedical applications. *J. Mater. Chem. B* **11**, 2036–2062 (2023). [doi:10.1039/D2TB02019J](https://doi.org/10.1039/D2TB02019J) [Medline](#)

37. D. Giordano, I. Boron, S. Abbruzzetti, W. Van Leuven, F. P. Nicoletti, F. Forti, S. Bruno, C.-H. C. Cheng, L. Moens, G. di Prisco, A. D. Nadra, D. Estrin, G. Smulevich, S. Dewilde, C. Viappiani, C. Verde, Biophysical characterisation of neuroglobin of the icefish, a natural knockout for hemoglobin and myoglobin. Comparison with human neuroglobin. *PLOS ONE* **7**, e44508 (2012). [doi:10.1371/journal.pone.0044508](https://doi.org/10.1371/journal.pone.0044508) [Medline](#)
38. H. P. Hersleth, T. Uchida, Å. K. Røhr, T. Teschner, V. Schünemann, T. Kitagawa, A. X. Trautwein, C. H. Görbitz, K. K. Andersson, Crystallographic and spectroscopic studies of peroxide-derived myoglobin compound II and occurrence of protonated FeIV O. *J. Biol. Chem.* **282**, 23372–23386 (2007). [doi:10.1074/jbc.M701948200](https://doi.org/10.1074/jbc.M701948200) [Medline](#)
39. T. H. Yosca, R. K. Behan, C. M. Krest, E. L. Onderko, M. C. Langston, M. T. Green, Setting an upper limit on the myoglobin iron(IV)hydroxide pK(a): Insight into axial ligand tuning in heme protein catalysis. *J. Am. Chem. Soc.* **136**, 9124–9131 (2014). [doi:10.1021/ja503588n](https://doi.org/10.1021/ja503588n) [Medline](#)
40. D. A. Svistunenko, N. A. Davies, M. T. Wilson, R. P. Stidwill, M. Singer, C. E. Cooper, Free radical in blood: A measure of haemoglobin autoxidation in vivo? †. *J. Chem. Soc., Perkin Trans. 2* 2539–2544 (1997). [doi:10.1039/A702483E](https://doi.org/10.1039/A702483E)
41. C. E. Cooper, D. J. Schaer, P. W. Buehler, M. T. Wilson, B. J. Reeder, G. Silkstone, D. A. Svistunenko, L. Bulow, A. I. Alayash, Haptoglobin binding stabilizes hemoglobin ferryl iron and the globin radical on tyrosine β 145. *Antioxid. Redox Signal.* **18**, 2264–2273 (2013). [doi:10.1089/ars.2012.4547.test](https://doi.org/10.1089/ars.2012.4547.test) [Medline](#)
42. Z. Ke, J. Chaudhary, L. Q. Flagg, K. N. Baustert, A. O. Yusuf, G. Liu, L. You, K. R. Graham, D. M. DeLongchamp, J. Mei, Controlled Dedoping and Redoping of N-Doped Poly(benzodifurandione) (n-PBDF). *Adv. Funct. Mater.* **34**, 2400255 (2024). [doi:10.1002/adfm.202400255](https://doi.org/10.1002/adfm.202400255)
43. P. Tiesinga, J.-M. Fellous, T. J. Sejnowski, Regulation of spike timing in visual cortical circuits. *Nat. Rev. Neurosci.* **9**, 97–107 (2008). [doi:10.1038/nrn2315](https://doi.org/10.1038/nrn2315) [Medline](#)
44. J. Veit, G. Handy, D. P. Mossing, B. Doiron, H. Adesnik, Cortical VIP neurons locally control the gain but globally control the coherence of gamma band rhythms. *Neuron* **111**, 405–417.e5 (2023). [doi:10.1016/j.neuron.2022.10.036](https://doi.org/10.1016/j.neuron.2022.10.036) [Medline](#)
45. H. E. Scharfman, The neurobiology of epilepsy. *Curr. Neurol. Neurosci. Rep.* **7**, 348–354 (2007). [doi:10.1007/s11910-007-0053-z](https://doi.org/10.1007/s11910-007-0053-z) [Medline](#)
46. M. C. Ridding, R. Inzelberg, J. C. Rothwell, Changes in excitability of motor cortical circuitry in patients with Parkinson's disease. *Ann. Neurol.* **37**, 181–188 (1995). [doi:10.1002/ana.410370208](https://doi.org/10.1002/ana.410370208) [Medline](#)
47. M. H. P. Kole, S. U. Ilschner, B. M. Kampa, S. R. Williams, P. C. Ruben, G. J. Stuart, Action potential generation requires a high sodium channel density in the axon initial segment. *Nat. Neurosci.* **11**, 178–186 (2008). [doi:10.1038/nn2040](https://doi.org/10.1038/nn2040) [Medline](#)
48. B. A. Bicknell, M. Häusser, A synaptic learning rule for exploiting nonlinear dendritic computation. *Neuron* **109**, 4001–4017.e10 (2021). [doi:10.1016/j.neuron.2021.09.044](https://doi.org/10.1016/j.neuron.2021.09.044) [Medline](#)

49. S. Xiao, S. Yadav, K. Jayant, Probing multiplexed basal dendritic computations using two-photon 3D holographic uncaging. *Cell Rep.* **43**, 114413 (2024). [doi:10.1016/j.celrep.2024.114413](https://doi.org/10.1016/j.celrep.2024.114413) [Medline](#)
50. P. Feyen, E. Colombo, D. Endeman, M. Nova, L. Laudato, N. Martino, M. R. Antognazza, G. Lanzani, F. Benfenati, D. Ghezzi, Light-evoked hyperpolarization and silencing of neurons by conjugated polymers. *Sci. Rep.* **6**, 22718 (2016). [doi:10.1038/srep22718](https://doi.org/10.1038/srep22718) [Medline](#)
51. N. Martino, P. Feyen, M. Porro, C. Bossio, E. Zucchetti, D. Ghezzi, F. Benfenati, G. Lanzani, M. R. Antognazza, Photothermal cellular stimulation in functional bio-polymer interfaces. *Sci. Rep.* **5**, 8911 (2015). [doi:10.1038/srep08911](https://doi.org/10.1038/srep08911) [Medline](#)
52. S. F. Owen, M. H. Liu, A. C. Kreitzer, Thermal constraints on in vivo optogenetic manipulations. *Nat. Neurosci.* **22**, 1061–1065 (2019). [doi:10.1038/s41593-019-0422-3](https://doi.org/10.1038/s41593-019-0422-3) [Medline](#)
53. A. R. Mardinly, I. A. Oldenburg, N. C. Pégard, S. Sridharan, E. H. Lyall, K. Chesnov, S. G. Brohawn, L. Waller, H. Adesnik, Precise multimodal optical control of neural ensemble activity. *Nat. Neurosci.* **21**, 881–893 (2018). [doi:10.1038/s41593-018-0139-8](https://doi.org/10.1038/s41593-018-0139-8) [Medline](#)
54. R. Prakash, O. Yizhar, B. Grewe, C. Ramakrishnan, N. Wang, I. Goshen, A. M. Packer, D. S. Peterka, R. Yuste, M. J. Schnitzer, K. Deisseroth, Two-photon optogenetic toolbox for fast inhibition, excitation and bistable modulation. *Nat. Methods* **9**, 1171–1179 (2012). [doi:10.1038/nmeth.2215](https://doi.org/10.1038/nmeth.2215) [Medline](#)
55. J. Vierock, S. Rodriguez-Rozada, A. Dieter, F. Pieper, R. Sims, F. Tenedini, A. C. F. Bergs, I. Bendifallah, F. Zhou, N. Zeitzschel, J. Ahlbeck, S. Augustin, K. Sauter, E. Papagiakoumou, A. Gottschalk, P. Soba, V. Emiliani, A. K. Engel, P. Hegemann, J. S. Wiegert, BiPOLES is an optogenetic tool developed for bidirectional dual-color control of neurons. *Nat. Commun.* **12**, 4527 (2021). [doi:10.1038/s41467-021-24759-5](https://doi.org/10.1038/s41467-021-24759-5) [Medline](#)
56. T. Kim, H. Kadji, A. J. Whalen, A. Ashourvan, E. Freeman, S. I. Fried, S. Tadigadapa, S. J. Schiff, Thermal effects on neurons during stimulation of the brain. *J. Neural Eng.* **19**, 056029 (2022). [doi:10.1088/1741-2552/ac9339](https://doi.org/10.1088/1741-2552/ac9339) [Medline](#)
57. D. Kim, J. W. Lee, S. Kang, W. Hong, J. Lee, H. J. Kwon, J. E. Jang, L. P. Lee, H. Kang, Simultaneous Detection of Neural Activity and Temperature in Photothermal Neural Stimulation, Simultaneous Detection of Neural Activity and Temperature in Photothermal Neural Stimulation. *Adv. Sci. (Weinh.)* **12**, e2411725 (2025). [doi:10.1002/advs.202411725](https://doi.org/10.1002/advs.202411725) [Medline](#)
58. S. A. Prescott, Y. De Koninck, Gain control of firing rate by shunting inhibition: Roles of synaptic noise and dendritic saturation. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 2076–2081 (2003). [doi:10.1073/pnas.0337591100](https://doi.org/10.1073/pnas.0337591100) [Medline](#)
59. D. B. Reichling, J. D. Levine, Heat transduction in rat sensory neurons by calcium-dependent activation of a cation channel. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7006–7011 (1997). [doi:10.1073/pnas.94.13.7006](https://doi.org/10.1073/pnas.94.13.7006) [Medline](#)
60. F. F. Weight, S. D. Erulkar, Synaptic transmission and effects of temperature at the squid giant synapse. *Nature* **261**, 720–722 (1976). [doi:10.1038/261720a0](https://doi.org/10.1038/261720a0) [Medline](#)

61. F. Salehpour, M. R. Hamblin, Photobiomodulation for Parkinson's disease in animal models: A systematic review. *Biomolecules* **10**, 610 (2020). [doi:10.3390/biom10040610](https://doi.org/10.3390/biom10040610) [Medline](#)
62. S. Xiao, n-PBDF neuromodulation dataset: Blood-Catalyzed n-Doped Polymers for Reversible Optical Neural Control, Zenodo (2025); <https://doi.org/10.5281/zenodo.17156036>.
63. S. Xiao, Code for data analysis: Blood-Catalyzed n-Doped Polymers for Reversible Optical Neural Control, Zenodo (2026); <https://doi.org/10.5281/zenodo.18434936>.
64. S. Xiao, Code for biophysical simulation: Blood-Catalyzed n-Doped Polymers for Reversible Optical Neural Control, Zenodo (2026); <https://doi.org/10.5281/zenodo.18435035>.
65. Scidraw, zebrafish silhouette, Zenodo (2019); <https://doi.org/10.5281/zenodo.3926120>.
66. E. Tyler, L. Kravitz, mouse drinking, Zenodo (2019); <https://doi.org/10.5281/zenodo.3925985>.
67. N. R. Eibergen, I. Im, N. Y. Patel, P. J. Hergenrother, Identification of a novel protein synthesis inhibitor active against gram-positive bacteria. *Chembiochem* **13**, 574–583, 490 (2012). [doi:10.1002/cbic.201100727](https://doi.org/10.1002/cbic.201100727) [Medline](#)
68. M. A. Hostetler, C. Smith, S. Nelson, Z. Budimir, R. Modi, I. Woolsey, A. Frerk, B. Baker, J. Gantt, E. I. Parkinson, Synthetic Natural Product Inspired Cyclic Peptides. *ACS Chem. Biol.* **16**, 2604–2611 (2021). [doi:10.1021/acscchembio.1c00641](https://doi.org/10.1021/acscchembio.1c00641) [Medline](#)
69. M. G. Shapiro, K. Homma, S. Villarreal, C.-P. Richter, F. Bezanilla, Infrared light excites cells by changing their electrical capacitance. *Nat. Commun.* **3**, 736 (2012). [doi:10.1038/ncomms1742](https://doi.org/10.1038/ncomms1742) [Medline](#)
70. P. Bankhead, M. B. Loughrey, J. A. Fernández, Y. Dombrowski, D. G. McArt, P. D. Dunne, S. McQuaid, R. T. Gray, L. J. Murray, H. G. Coleman, J. A. James, M. Salto-Tellez, P. W. Hamilton, QuPath: Open source software for digital pathology image analysis. *Sci. Rep.* **7**, 16878 (2017). [doi:10.1038/s41598-017-17204-5](https://doi.org/10.1038/s41598-017-17204-5) [Medline](#)
71. P. Spangenberg, N. Hagemann, A. Squire, N. Förster, S. D. Krauß, Y. Qi, A. Mohamud Yusuf, J. Wang, A. Grüneboom, L. Kowitz, S. Korste, M. Totzeck, Z. Cibir, A. A. Tuz, V. Singh, D. Siemes, L. Struensee, D. R. Engel, P. Ludewig, L. Martins Nascentes Melo, I. Helfrich, J. Chen, M. Gunzer, D. M. Hermann, A. Mosig, Rapid and fully automated blood vasculature analysis in 3D light-sheet image volumes of different organs. *Cell Rep. Methods* **3**, 100436 (2023). [doi:10.1016/j.crmeth.2023.100436](https://doi.org/10.1016/j.crmeth.2023.100436) [Medline](#)
72. H. F. Khan, S. Dutta, A. N. Scott, S. Xiao, S. Yadav, X. Chen, U. K. Aryal, T. L. Kinzer-Ursem, J.-C. Rochet, K. Jayant, Site-specific seeding of Lewy pathology induces distinct pre-motor cellular and dendritic vulnerabilities in the cortex. *Nat. Commun.* **15**, 10775 (2024). [doi:10.1038/s41467-024-54945-0](https://doi.org/10.1038/s41467-024-54945-0) [Medline](#)
73. M. Krzywinski, N. Altman, P. Blainey, Points of significance: Nested designs. For studies with hierarchical noise sources, use a nested analysis of variance approach. *Nat. Methods* **11**, 977–978 (2014). [doi:10.1038/nmeth.3137](https://doi.org/10.1038/nmeth.3137) [Medline](#)
74. A. Y. Hsu, T. Wang, R. Syahirah, S. Liu, K. Li, W. Zhang, J. Wang, Z. Cao, S. Tian, S. Matosevic, C. J. Staiger, J. Wan, Q. Deng, Rora Regulates Neutrophil Migration and

Activation in Zebrafish. *Front. Immunol.* **13**, 756034 (2022).

[doi:10.3389/fimmu.2022.756034](https://doi.org/10.3389/fimmu.2022.756034) [Medline](#)

75. T. Lei, J.-H. Dou, X.-Y. Cao, J.-Y. Wang, J. Pei, Electron-deficient poly(p-phenylene vinylene) provides electron mobility over $1 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ under ambient conditions. *J. Am. Chem. Soc.* **135**, 12168–12171 (2013). [doi:10.1021/ja403624a](https://doi.org/10.1021/ja403624a) [Medline](#)