Supporting Information

Supramolecular peptidic dopants for inducing photoconductivity and mechanical tunability in digital light processable hydrogels

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1. Materials and Methods

Peptide synthesis. Amino acids and Wang-Asp(OtBu) resin were obtained from Advanced ChemTech. 4,4′,4″,4‴-(porphine-5,10,15,20-tetrayl)tetrakis(benzoic acid) (porphyrin) was obtained from Millipore-Sigma. *O*-(benzotriazole-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), trifluoroacetic acid (TFA), triisopropylsilane (TIPS), and *N,N*-diisopropylethylamine (DIPEA) were obtained from Oakwood Chemical. Dicholoromethane, *N*-methyl-2-pyrrolidinone (NMP), methanol, *N,N*-dimethylformamide (DMF), isopropanol (IPA), acetonitrile, ethyl ether, hexanes, ammonium hydroxide, and potassium hydroxide (KOH) were obtained from Fisher Scientfic. DIPEA was degassed by sparging with nitrogen gas (N_2) over 4 Å molecular sieves for approximately 1 hour.

Peptides synthesis was performed both manually and with the Liberty Blue microwave peptide synthesizer. Manual synthesis was performed for DGRDK$_a$VVD from the allyloxycarbonyl-terminated Lys (K$_a$; K-alloc) position onwards using a peptide chamber. Fmoc-deprotection was performed using 20 vol% piperidine in DMF. After swelling initially in DCM, deprotection solution was added twice, shaking for 2 and 10 minutes, followed by washing with NMP, methanol, and DCM in the respective order 3 times. Amino acid solutions were prepared with a 1:3 equivalence of resin to amino acid, along with 2.9 equiv. HBTU and 10 equiv. DIPEA, all fully dissolved via sonication in 10 mL NMP. The amino acid solution was added to the peptide chamber and shaken for 1 hour. Following the coupling, resin was washed with NMP, methanol, and DCM in the respective order 3 times. Each coupling was confirmed using a Kaiser test, with amino acid couplings being repeated as needed.

Porphyrin coupling the *N*-terminus of the peptide backbone. The porphyrin coupling to peptides was adapted from Sanders et al. (*ACS Omega* **2017**, *2* (2), 409-419). Fmoc-deprotection of the resin was performed using 20 vol% piperidine in DMF. After swelling initially in DCM, deprotection solution was added twice, shaking for 2 and 10 minutes, followed by washing with NMP, methanol, and DCM in the respective order 3 times. Using a mmol basis of 0.05 resin, porphyrin 0.075 mmol was dissolved in 10 mL NMP with PyBOP (0.33 mmol) and DIPEA (4.5 mmol). The porphyrin cocktail was fully dissolved via sonication and added to the Fmoc-deprotected resin, mixing for 15 hrs. After the first coupling, resin was washed (NMP 3x, DMF

3x, IPA 2x, water 2x, IPA 4x, ACN 2x, ether 2x, hexanes 2x). The resin was re-swelled in DCM and coupled with porphyrin (0.05 mmol), PyBOP (0.22 mmol), and DIPEA (3.0 mmol) fully dissolved in NMP via sonication. The second coupling was performed for 24 hrs, followed by the same wash procedure.

Acid cleavage of functionalized peptides from resin. Upon completion of the porphyrin couplings, resin was treated with a trifluoroacetic acid cocktail (9.5 mL TFA, 250 µL water, 250 µL TIPS) for 2-3 hours. A vertical orbital shaker was used to maintain shaking of the peptide chamber with the acid cocktail. The peptide solution was collected, and resin was washed 3x with DCM. The solution was condensed under reduced pressure. Crude peptide was collected by adding chilled ethyl ether (~ 30 mL) and centrifuging the solution (10 min, 300 rpm) to isolate the peptide, followed by decanting the ether. The crude product, with peptide-porphyrin conjugates, was fully dissolved in water with a small amount of ammonium hydroxide and lyophilized afterwards.

Peptide-porphyrin conjugate purification. Reverse phase high-performance liquid chromatography (RP-HPLC) was performed (Agilent Infinity II Preparative HPLC System) to purify peptide-porphyrin conjugates after synthesis. Acetonitrile and 0.1 vol% ammonium formate (pH 10) were used as the two mobile phase buffers. A ZORBAX C8 column (Zorbax Eclipse XDB-C8, 21.2 x 250 mm) was used for purification. Purity evaluation was performed via analytical HPLC.

Mass spectrometry (MS). Several MS techniques were performed to confirm peptide mass and purity. MALDI-TOF was performed using the ABSciex. LC-MS was performed using the Waters Acquity UPLC-QDA and Xevo G2-XS QTOF.

Preparation of assembled porphyrin-peptide conjugates. Porphyrin-peptide assembly was standardized based on mass and volume. Solutions were prepared to be 100 µL of 1 mg/mL porphyrin-peptide conjugate in water. Solutions were titrated to pH 8 with 1 M KOH or ammonium hydroxide. For acid assembly, 10 µL of 1 M HCl was added to the porphyrin-peptide aliquot and mixed well. For salt assembly, 10 µL of 200 mM $CaCl_2$ was added to the porphyrin-peptide aliquot and mixed well.

Transmission electron microscopy (TEM). Samples were prepared on 200 mesh formvar coated carbon grids with a 4 nm carbon coating (Electron Microscopy Services) for TEM imaging. To

prepare the sample, 100 µL of 1 mg/mL solution was made and 10 µL of the respective assembly trigger (1 M HCl, 200 mM $CaCl_2$) was added to the solution. The carbon face of the grids was glow discharged at 10 mA for 69 seconds. A small amount of peptide solution (~5 uL) was deposited onto the carbon face for 60 seconds. Grids were washed with Milli-Q water three times and dried with filter paper, then stained with 1% uranium acetate for 30 seconds and dried. TEM imaging was performed using a JEOL JEM-2100F equipped with a CCD camera, Gatan K3 direct electron detector, and a Gatan OneView camera at an operating voltage of 200 kV. Grids were imaged in a single-tilt holder. Images were acquired and processed using DigitalMicrograph.

Ultraviolet-Visible (UV-Vis) absorption spectroscopy. UV-Vis absorption spectra of porphyrin-peptide conjugates were recorded using the Cary 100 UV-vis spectrophotometer. Samples were prepared as 3 mL of 1 mg/mL solutions using Milli-Q water and titrated with 1 M KOH to pH ~8. 1 M HCl was used for acidification and 200 mM $CaCl_2$ was used for calcium assembly. Porphyrin-peptide solutions were mixed before recording UV-vis spectra. A cuvette with 1 mm pathlength was used for measurements.

Photoluminescence (PL) spectroscopy. PL spectra were recorded using the Cary Eclipse fluorescence spectrometer. Samples were prepared using the procedure for UV-vis measurements. excited with respect to the maximum absorption wavelength. The excitation and emission slit widths were set to 10 nm.

Circular dichroism (CD) spectroscopy. CD spectra were recorded using the Jasco-810 spectropolarimeter. Samples were prepared using the procedure for UV-vis absorbance measurements.

Digital light processing (DLP) printing of peptide-PEGDA hydrogels. 3-D printing was performed using the CELLINK BIONOVA X. Peptide solutions were made at 10 mg/mL using DNase/RNase-free distilled water (Fisher Scientific). PEGDA 3.4K (Advanced Biomatrix) was dissolved in UltraPure water to make a 30 wt% solution. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, Cellink) was dissolved in UltraPure water to make a 5 wt% stock solution. PEGDA, LAP, and water were combined in a 24-well glass bottom plate (Cellvis). The peptide sample was prepared adjacently before mixing with the PEGDA + LAP solution. For peptide gels, a 10 mg/mL peptide-porphyrin solution in water was prepared. An aliquot of peptide and trigger (water blank, 1 M HCl, 200 mM $CaCl_2$) was prepared, with water replacing the peptide

solution for negative controls. After mixing the peptide aliquot well, it was added to the PEGDA + LAP mix in the 24-well glass bottom plate. Peptide-PEGDA solutions were standardized to be composed of 1 mg/mL peptide-porphyrin, 20 wt% PEGDA 3.4K, 0.5 wt% LAP, and UltraPure water. All gels were printed at 25℃ using a 24-well glass bottom plate and probe (CELLINK). The design used for printing gels for rheology measurements was an 8 mm diameter cylinder with 0.5 mm height. For printability assessment, the Ardoña lab logo was converted from an image to STL using InkScape. Monomer and calcium-assembled peptide-PEGDA gels were printed using 60% light intensity and a print speed of 0.005 mm/s. Acid-assembled peptide-PEGDA gels were printed using 100% light intensity and 0.002 mm/s. Upon fabrication, gels were washed with and stored in UltraPure water. Gels printed for rheology measurements were stored in UltraPure water overnight at 4°C before testing.

Rheology of peptide-PEGDA hydrogels. Rheology was measured using the TA DHR-2 rheometer in the TEMPR facility at UC Irvine. An 8 mm cross hatched plate (TA Instruments) was used for the upper geometry and a standard Peltier plate was used for the lower plate. All experiments were performed at a temperature of 25℃. Amplitude sweeps were performed at 1 Hz from 0.01-1000% strain with 5 points/decade. Frequency sweeps were performed at 1% strain from 0.1-100 Hz with 5 points/decade. Stress relaxation tests were performed at 1% strain for 15 minutes. For frequency sweeps and stress relaxation tests, the gel was surrounded with water to prevent drying.

Conductivity and photoconductivity assessment of peptide films and gels. Photoconductivity was measured using the Keithley 4200 SCS semiconductor parameter analyzer from Dr. Camilo Cuervo at UC Irvine. To make peptide films, 100 µL of 1 mg/mL solution was prepared and 10 µL of the respective assembly trigger (1 M HCl, 200 mM $CaCl_2$) was mixed into the diluted aliquot. Assembled peptide solution was deposited onto a silicon wafer to cover the entire surface, and wafers were left to dry overnight. Hydrogels were prepared following the bioprinting protocol. To prepare wafers for measurement, two probes were placed on different devices within a grid. For hydrogel photoconductivity, gels were submerged in Tyrode's solution while a silver chloride (AgCl) probe was placed into the solution and a platinum probe was inserted into the gel. For conductivity measurements, a current of 30 V was applied. For photocurrent measurements, 0.1 V was applied and a 415 nm light source was used with a frequency of 2 Hz for light pulsing.

2. MS Characterization of Peptide-Porphyrin Conjugates

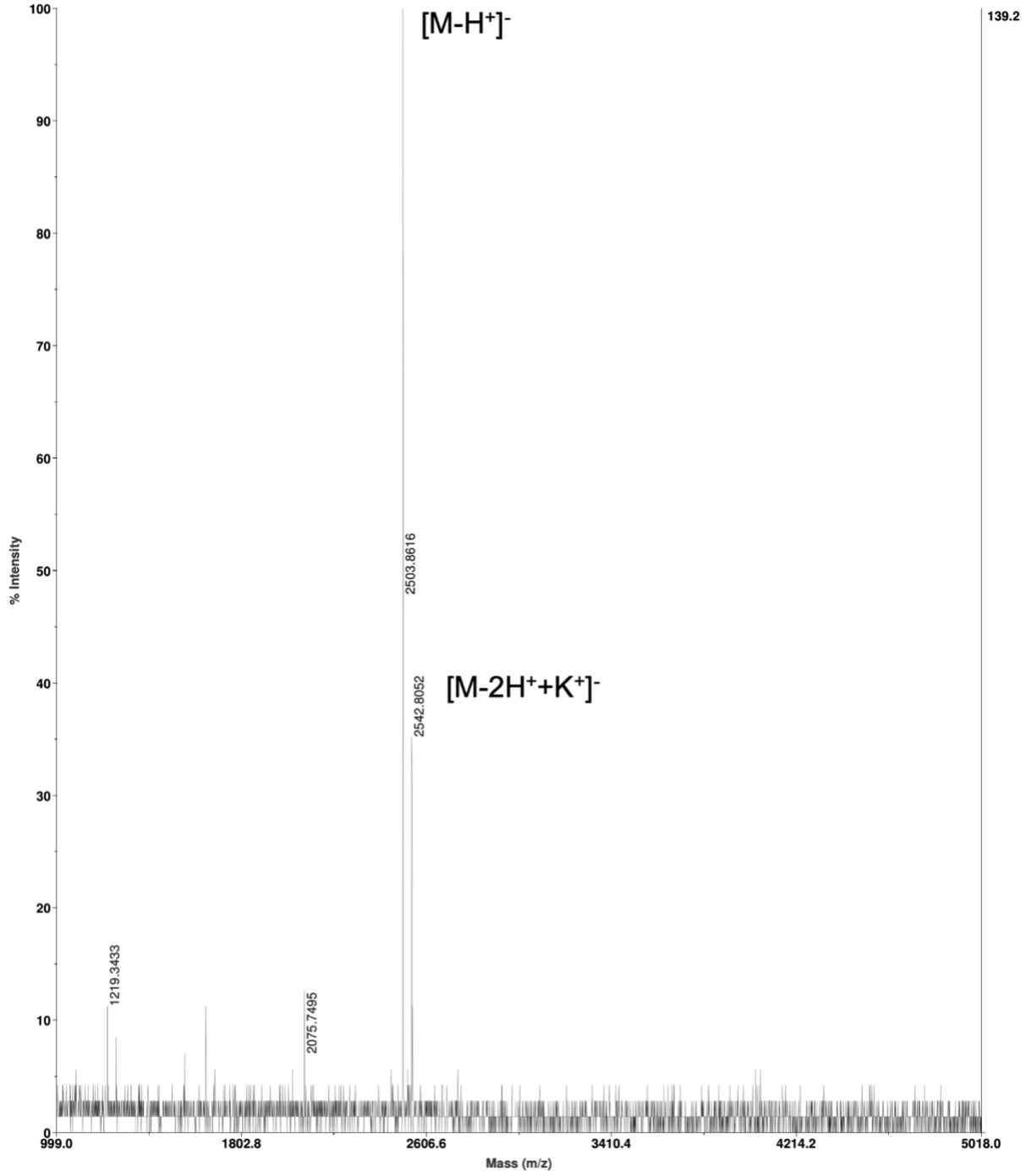

Figure S1. MS spectrum (from MALDI-TOF/TOF) of DVVD-porphyrin.

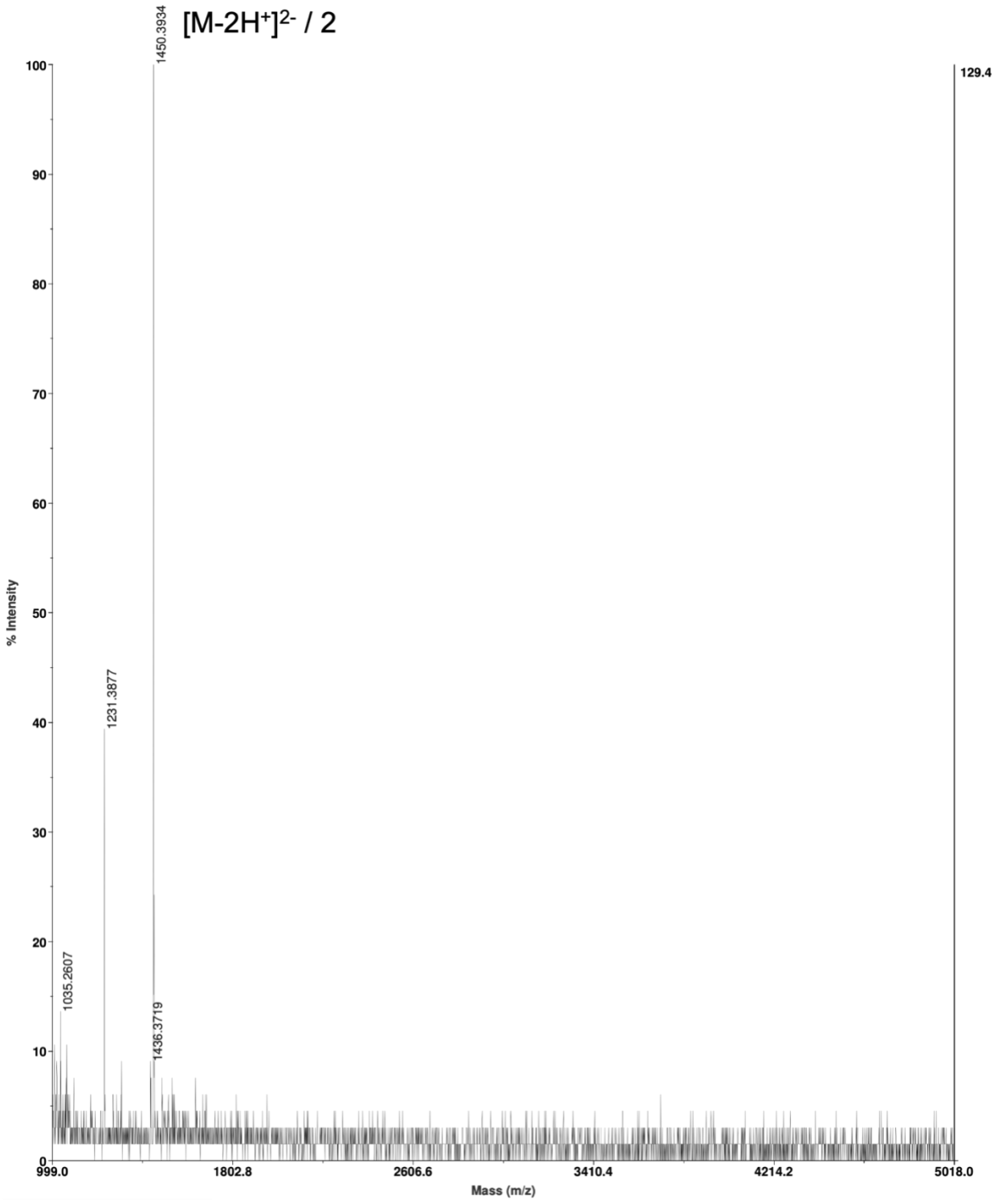

Figure S2. MS spectrum (from MALDI-TOF/TOF) of DFFD-porphyrin.

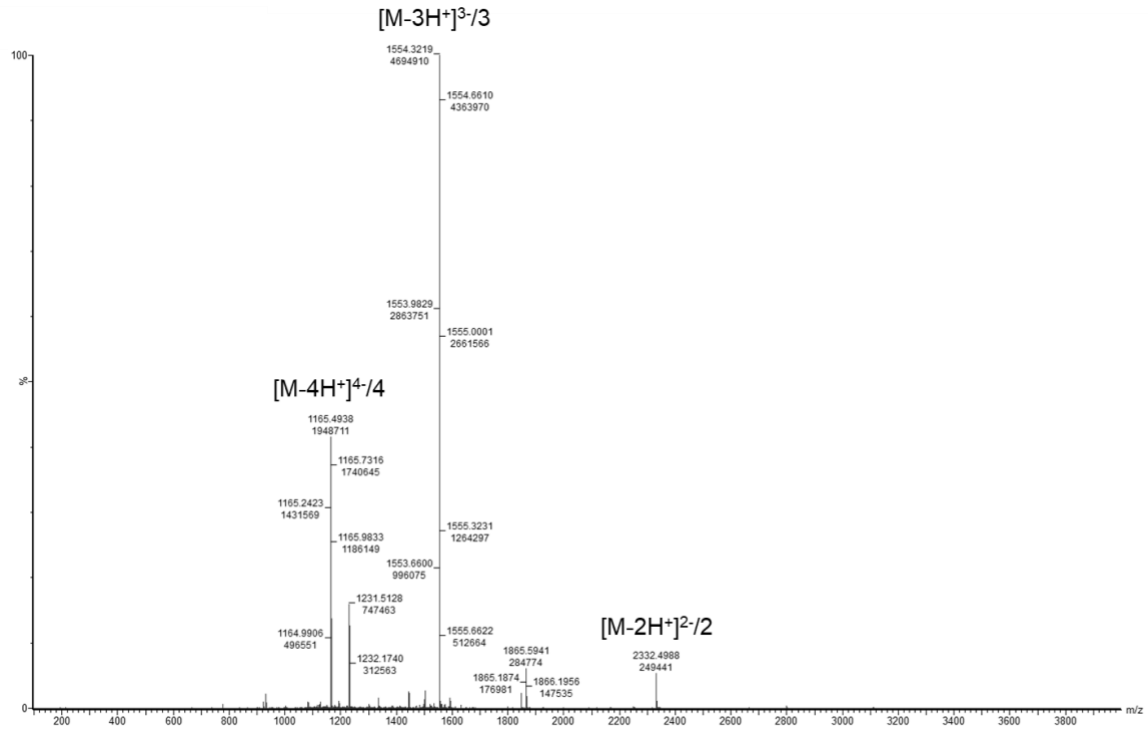

Figure S3. MS spectrum (from LC-MS scan) of DGRDK$_a$VVD-porphyrin.

3. HPLC Characterization of Peptide-Porphyrin Conjugates

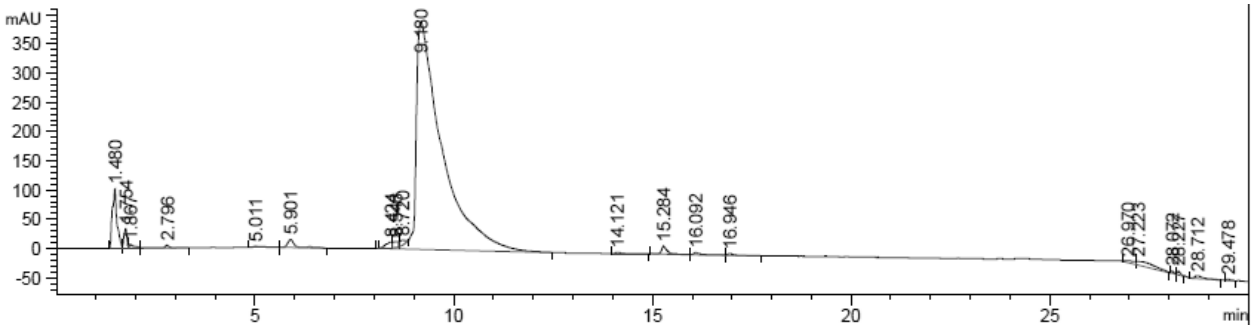

Figure S4. HPLC trace of purified DVVD-porphyrin, monitored at 220 nm.

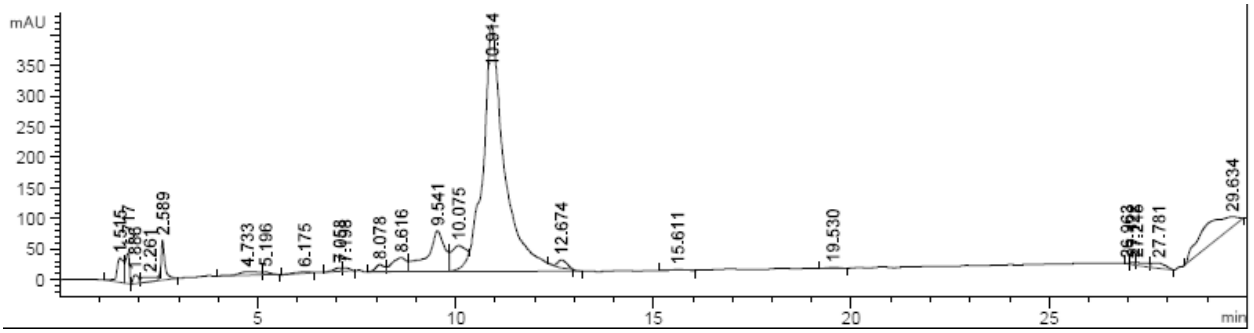

Figure S5. HPLC trace of purified DFFD-porphyrin, monitored at 220 nm.

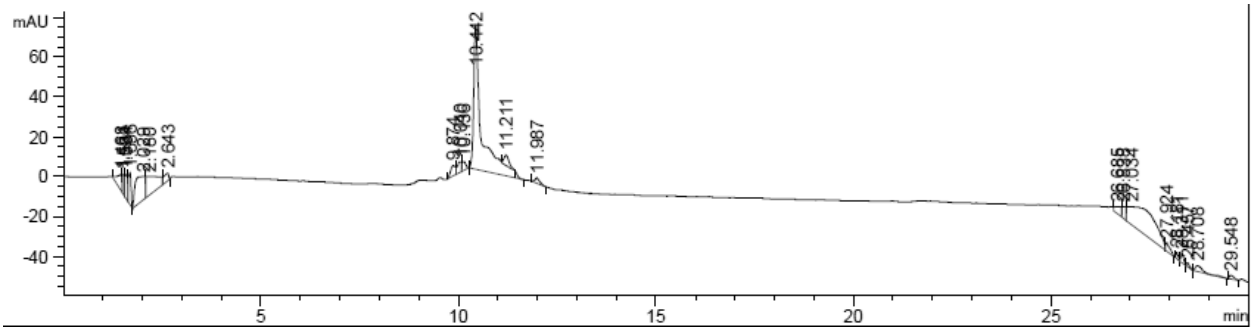

Figure S6. HPLC trace of purified DGRDK$_a$VVD-porphyrin, monitored at 220 nm.

5. Circular Dichroism of Peptide-Porphyrin Conjugates

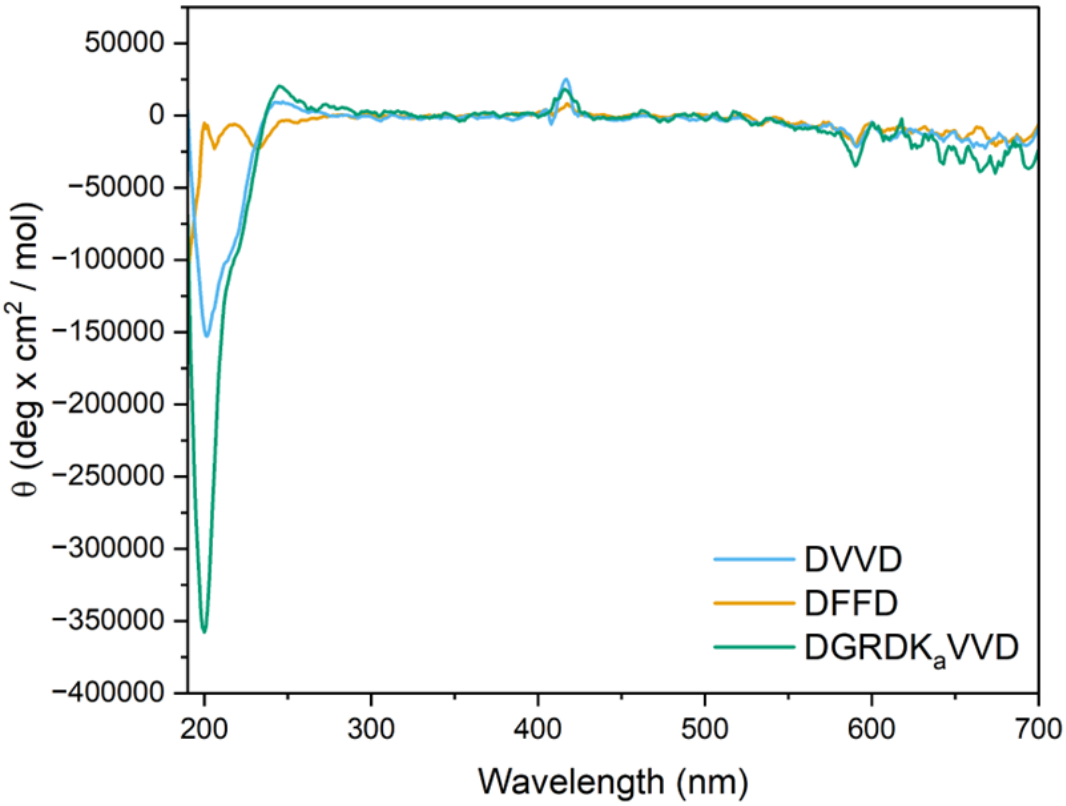

Figure S7. Circular dichroism of peptide-porphyrin conjugates in non-assembled/molecularly dispersed state (pH 8).

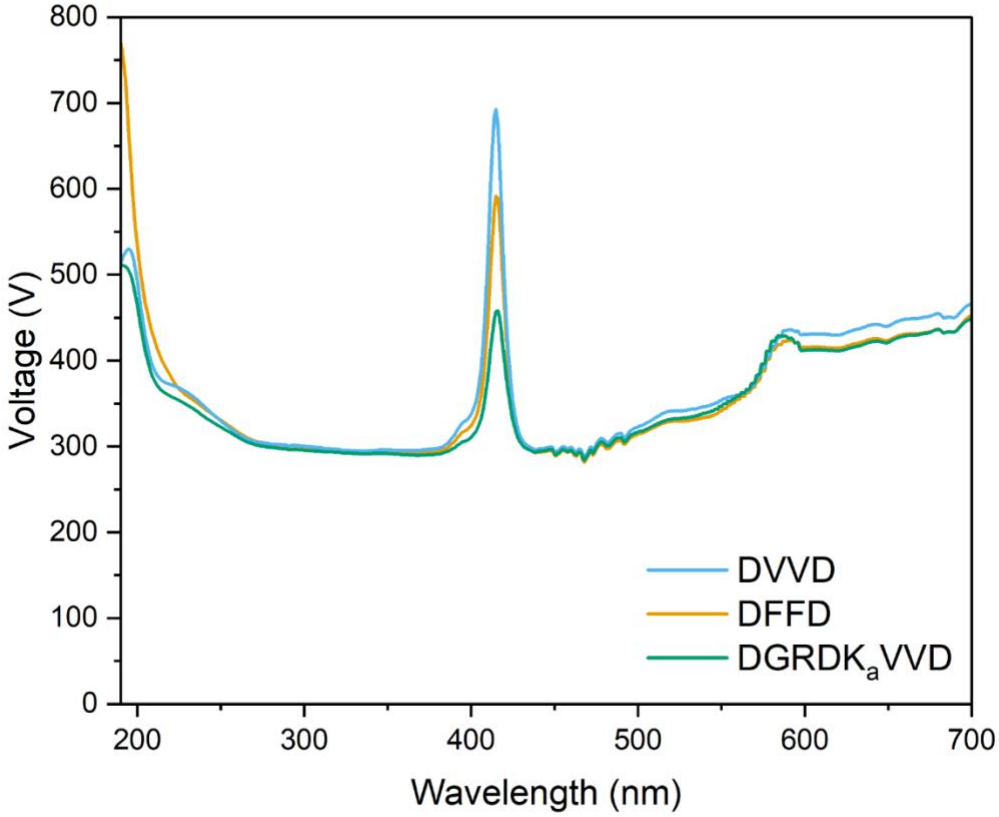

Figure S8. Dynode voltage vs. wavelength plots for the CD spectra in Figure S7.

6. Rheology Characterization of Peptide-PEGDA Hydrogels

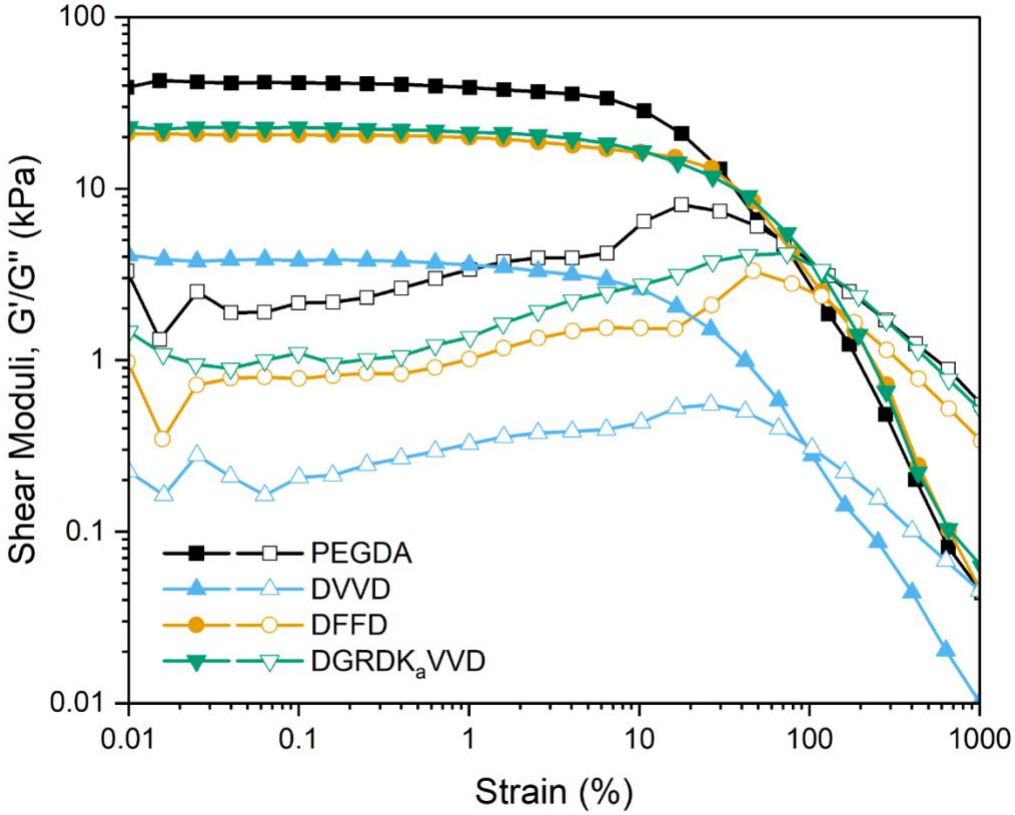

Figure S9. Representative amplitude sweep of peptide-PEGDA hydrogels in dispersed state (pH 8) performed at 1 Hz. Solid shapes = storage modulus, G′; hollow shapes = G″, loss modulus.

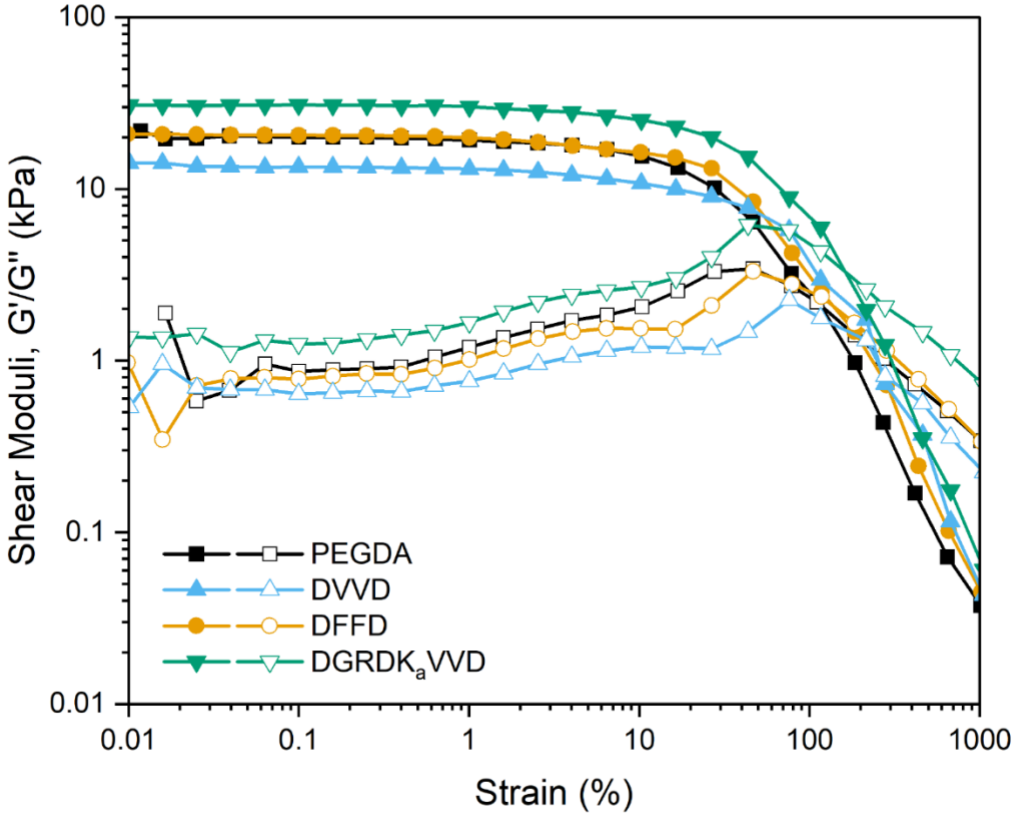

Figure S10. Representative amplitude sweep of peptide-PEGDA hydrogels at pH 2 performed at 1 Hz. Solid shapes = storage modulus, G′; hollow shapes = G″, loss modulus.

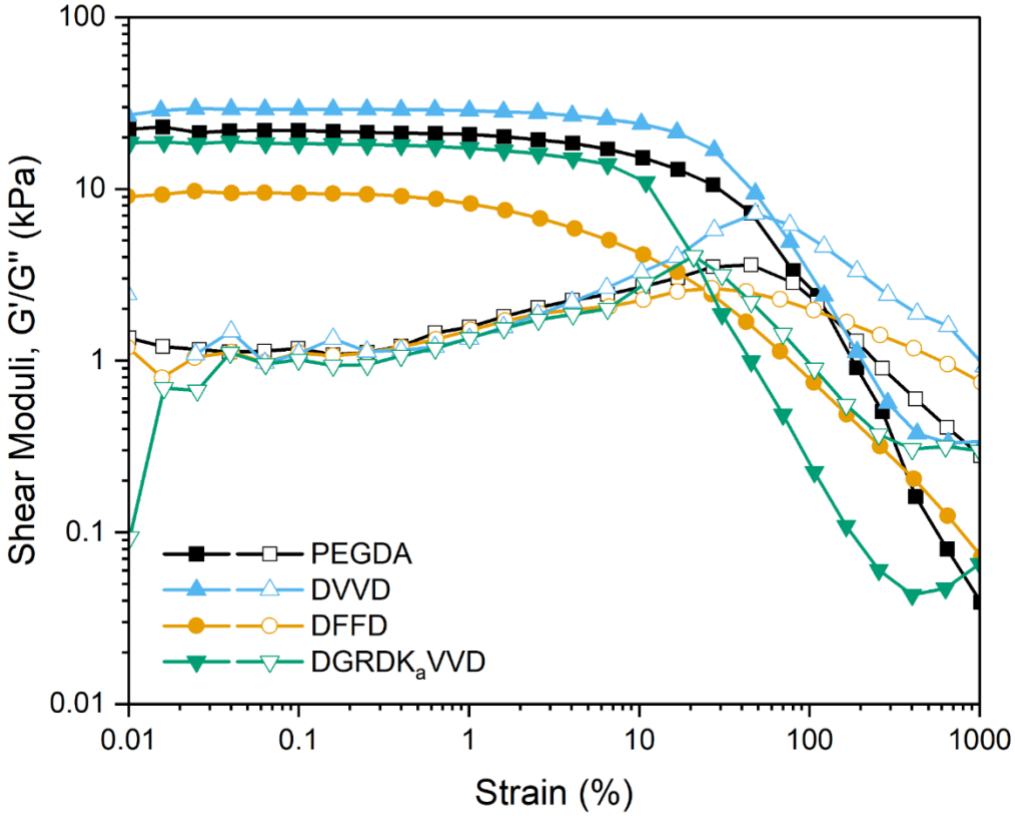

Figure S11. Representative amplitude sweeps of peptide-PEGDA hydrogels with $CaCl_2$ performed at 1 Hz. Solid shapes = storage modulus, G′; hollow shapes = G″, loss modulus.

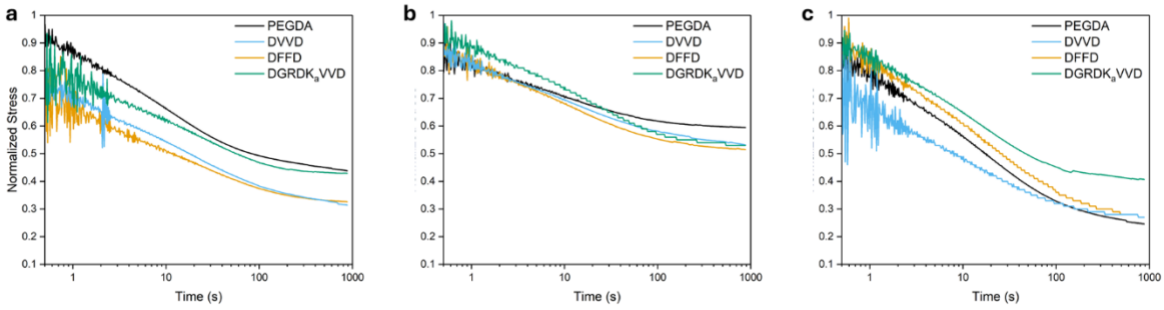

Figure S12. Stress relaxation of peptide-PEGDA hydrogels in dispersed state (pH 8) (a), pH 2 (b), $CaCl_2$-triggered (c).

7. Conductivity and Photoconductivity Measurements of Films and Hydrogels

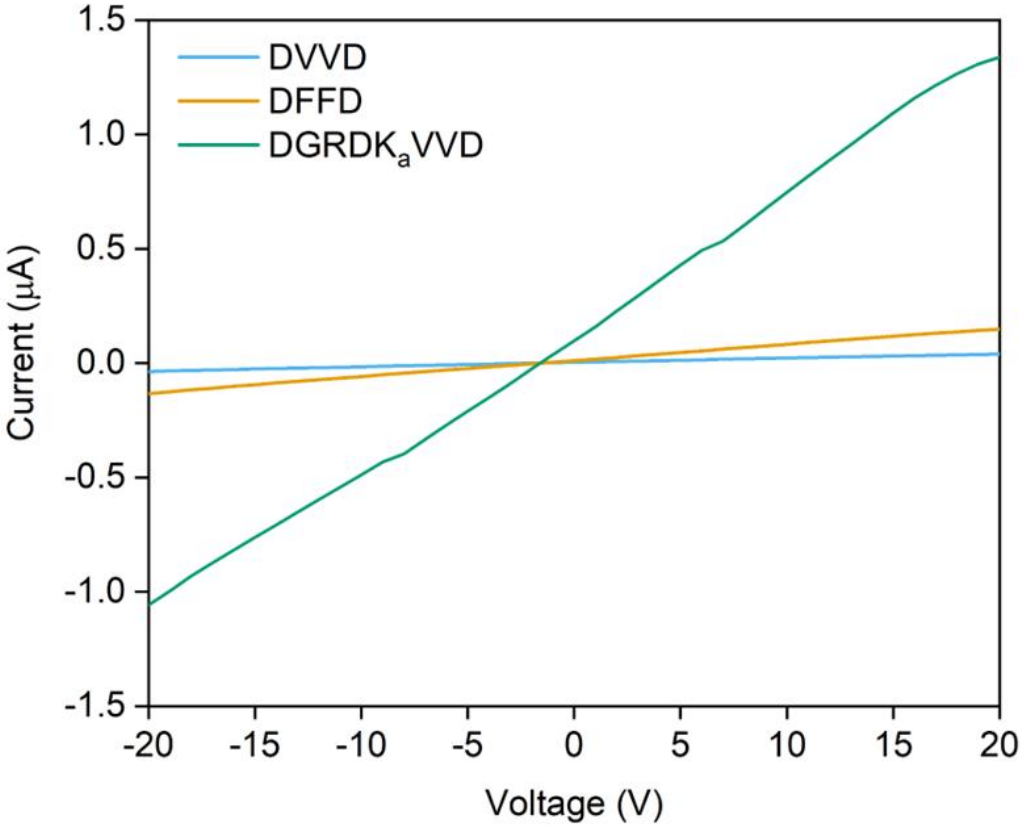

Figure S13. Representative current-voltage (I-V) curve of acid-assembled (pH 2) porphyrin-peptide films.

8. Printing Parameters Screening: Representative Images of Resulting Gels

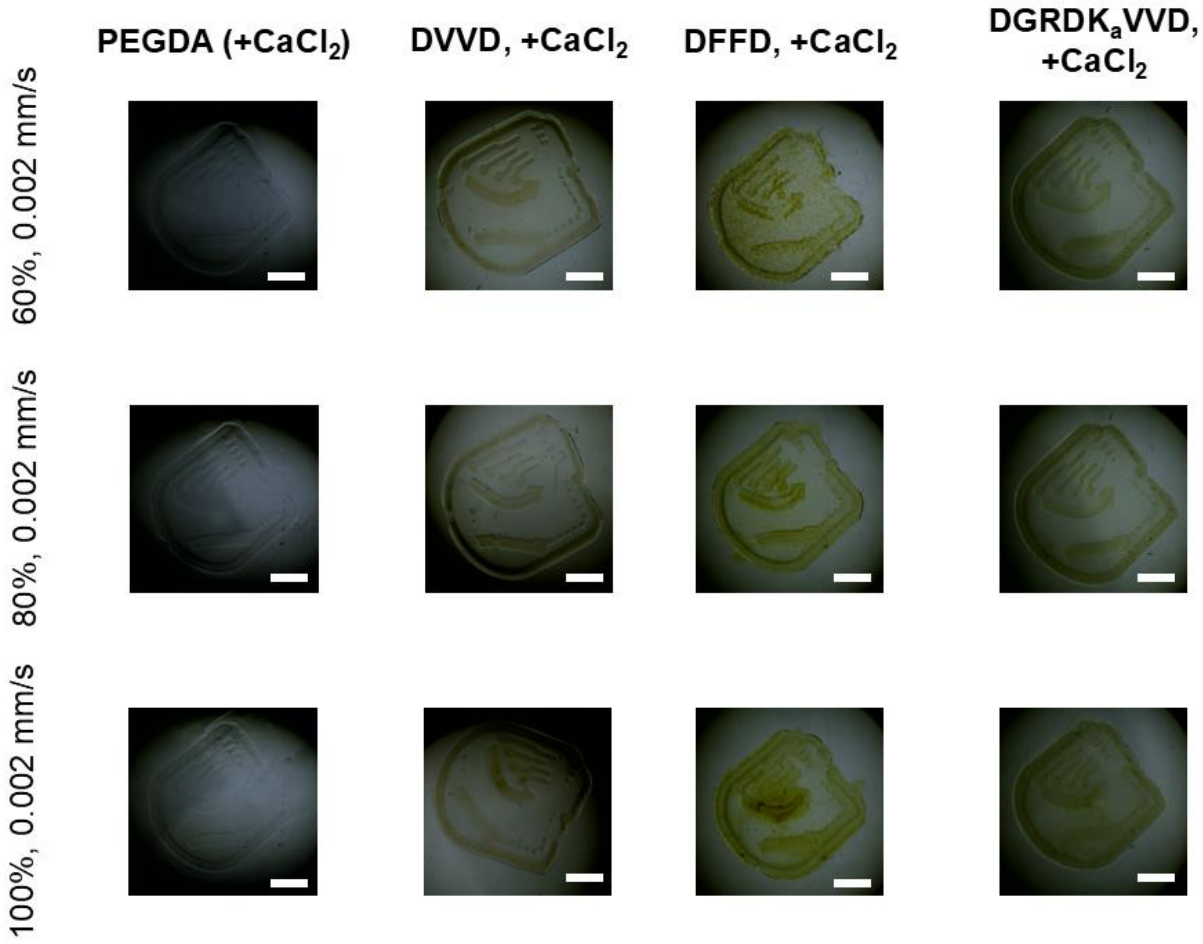

Figure S14. Stereoscope images of peptide-PEGDA hydrogels with $CaCl_2$ trigger. Parameters listed on the left detail the light intensity and print speed used for DLP printing. Scale bar = 2 mm.

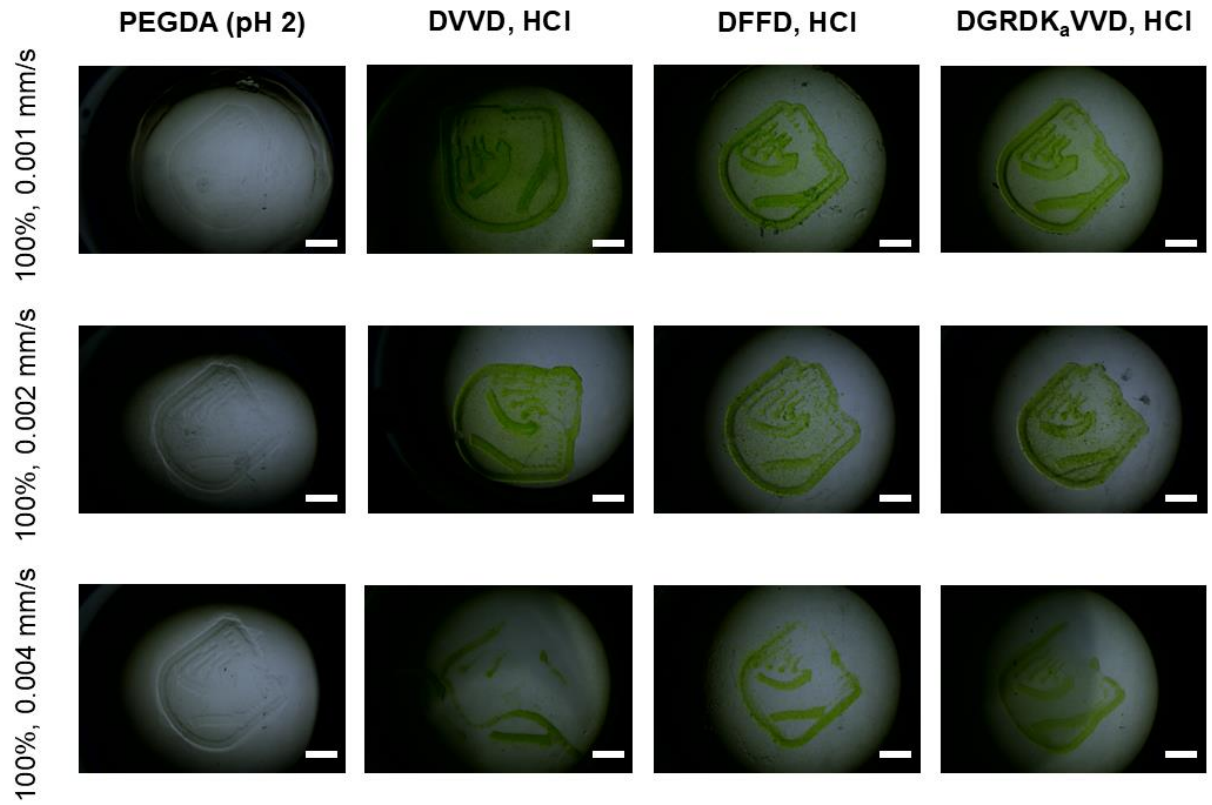

Figure S15. Stereoscope images of peptide-PEGDA hydrogels at pH 2. Parameters listed on the left detail the light intensity and print speed used for DLP printing. Scale bar = 2 mm.