Electronic Supplementary Material (ESI) for Soft Matter.

The Tripeptide GHG as an Unexpected Hydrogelator Triggered by Imidazole Deprotonation

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Electronic Supporting Information

Experimental

Material

Glycyl-histidyl-glycine (H-Gly-His-Gly-OH) was purchased from Bachem with >99% purity and used without further purification. Peptides were provided without TFA. Deionized water was used for sample preparation. pH was adjusted using sodium hydroxide (NaOH) (50 w/w% in water, Aldrich) and hydrogen chloride (HCl) (50 v/v% in water, Ricca). Deuterated water (D_2O) (99.9% purity, Aldrich), deuterium chloride (DCl) (35 wt% in D_2O, 99 atom% D, Aldrich), and sodium deuteroxide (NaOD) (30 wt% in D_2O, 99 atom% D, Aldrich) were used for vibrational spectroscopy. All samples were initially dissolved in acid to pH between 2 and 3, then adjusted to the pH of interest using base. The pH of the samples was measured at ambient temperature prior to the formation of visible aggregation by a Fisher Scientific Accument AR50 pH probe, software revision 1.04b.

Spectroscopy

Ultra-violet electronic circular dichroism (UVCD) spectra were measured on a Jasco J-810 spectropolarimeter (model J-810-150S) purged with nitrogen. The temperature was maintained using a Peltier controller (model PTC-423S). The sample was loaded onto a 50 µm cell from International Crystal Laboratories. UVCD spectra were recorded between 180 and 300 nm with a 500 nm/min scan speed, 1 s response time, 0.05 nm data pitch, and 5 nm bandwidth. Ten spectra were averaged at each time interval, and all spectra were background corrected.

Vibrational circular dichroism and Fourier transform infrared (VCD/FTIR) spectra were measured on a BioTools ChiralIR and were loaded in a 121 µm CaF_2 biocell. Spectra were collected with a resolution of 8 cm^{-1} and scan speed of 83 scans per minute using the Grams/IR 7.00 software (Thermo Galactic). Spectra were collected over one hour. The sample temperature was maintained at room temperature by a BioTools water-cooled temperature controller. The IR spectra were not solvent corrected. Part of the observed spectrum was decomposed into individual Gaussian (amide I region) and Voigtian bands (COO^- as) using the MULTIFIT software.

Rheology

Rheology measurements were obtained on a DHR-3 (TA instruments) using a Peltier plate for temperature control with a top plate of diameter 25 mm. Around 400 µL of peptide material was used. To avoid solvent evaporation, safflower oil was added as a solvent trap around the free surface of the sample to avoid evaporation. The samples were prepared one to three minutes prior to loading. The exact time between sample preparation and the beginning of the experiment was recorded and accounted for during data treatment. A Peltier plate was used to maintain a constant 20 °C temperature throughout the experiment. A gap of 700 µm was used for all steps. The mechanical properties of the hydrogel were probed by small amplitude oscillatory shear measurements. We performed an angular frequency sweep from 100 rad s^{-1} to 10^{-3} rad s^{-1} with a 0.03 % strain.

Microscopy

The GHG gels were observed using with an Amscope 7X-45X Trinocular Stereo Zoom microscope equipped with an Amscope MU130 camera.

Wide-Angle X-ray scattering

The WAXS experiments were carried out on a Xenocs Xeuss 2.0 diffractometer operated at a voltage of 50 kV and a current of 0.6 mA equipped with a Cu Kα X-ray source (Xenocs Genix3D Cu ULD) with a 1.54 Å wavelength. A Dectris Pilatus 1M solid state detector was used. The distance between the sample and detector was 165 mm. A set of slits controlled the beam focal spot which was adjusted to 0.7 mm x 0.7 mm. The samples were placed on a plate mounted onto an X–Z scanning motor. An initial scan was run to find the minimum transmitted intensity that corresponds to the highest density of material. Each sample was exposed for 10 min. The obtained 2D diffraction patterns were analyzed using the Foxtrot software. A silver behenate sample was used to calibrate the measurements. In order to obtain the integrated spectrum of the gel the contribution from the amorphous fraction of the sample was subtracted.

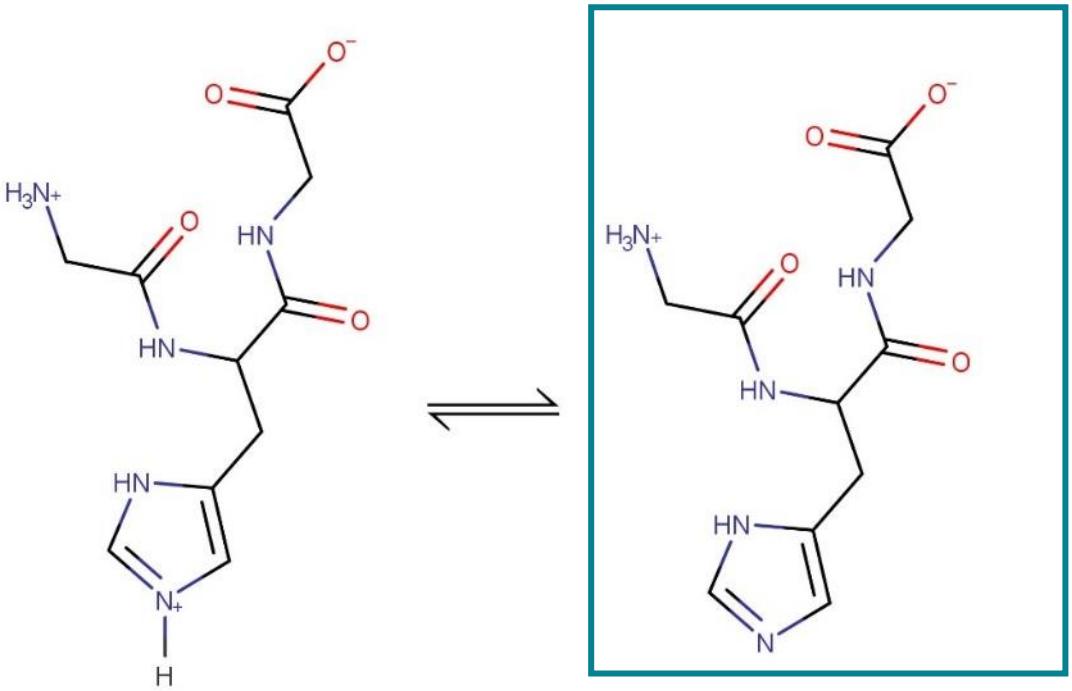

Figure S1: Chemical structure of two protonation states of glycylhistidylglycine. On the left the C-terminal carboxylate group is deprotonated while the imidazole group is still fully protonated. On the right (framed) the imidazole group is protonated and the peptide exhibits a zwitterionic state. In this state the peptide can self-assemble into the crystalline fibrils shown in Figure 2 of the main manuscript.

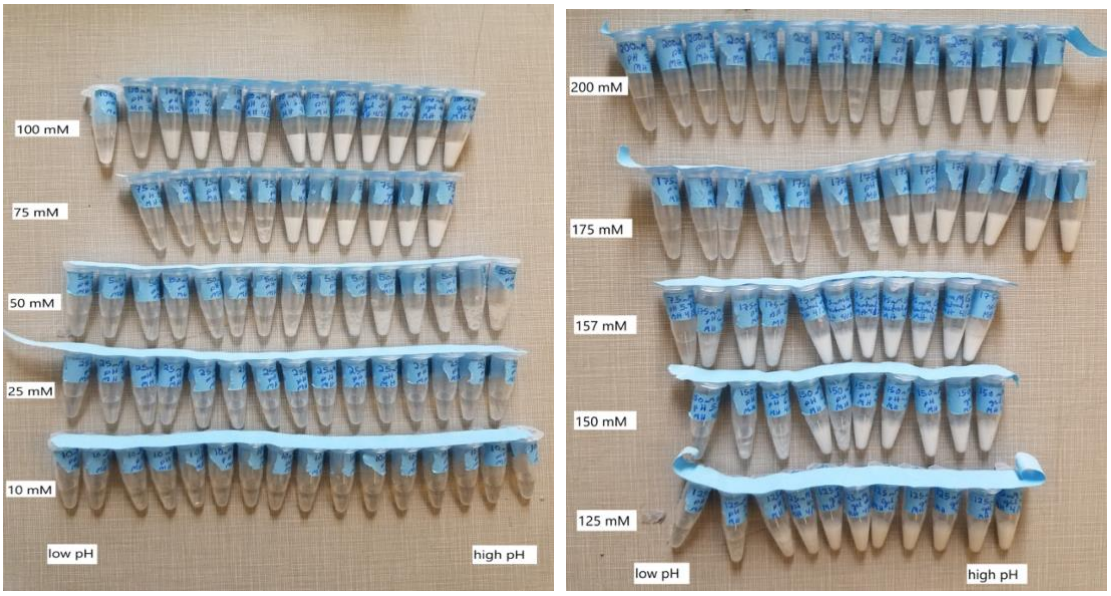

Figure S2: Photo images of the GHG samples used to obtain the phase diagram in Figure 1. The respective peptide concentrations are indicated. The number of samples shown exceed the number of data points in Figure 1 because the pH value could not accurately be determined. The turbid samples were all obtained in the green region of the phase diagram. However, the number of turbid samples shown exceed the number of data points in the green region of Figure 1 because the pH value could not accurately be determined.

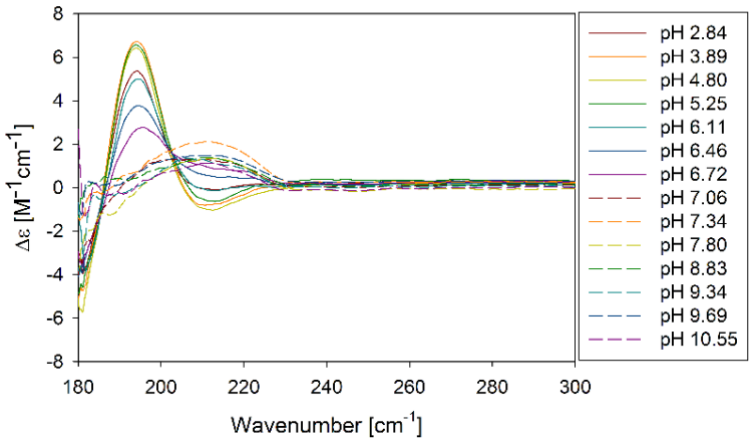

Figure S3. UVCD spectra of 10 mM GHG in water measured at the indicated pH-values in a region between 2.8 and 10.6. The spectra clearly display an isodichroic point.

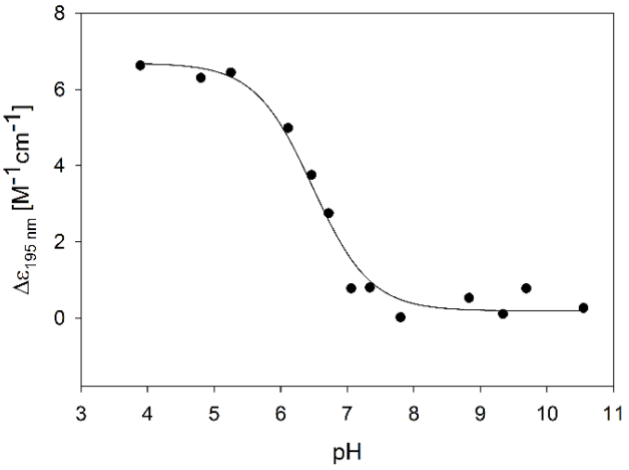

Figure S4. UVCD of 10 mM GHG in water measured at 195 nm plotted as a function of pH. The solid line was obtained by fitting the Henderson-Hasselbach type equation $\Delta\epsilon = \frac{\Delta\epsilon^{+}_{215} * K * 10^{-pH} + \Delta\varepsilon_{215}}{1 + K * 10^{-pH}}$ to the experimental data where $\mathrm{D}e^{+}_{215}$ and $\mathrm{D}e_{215}$ are the dichroism values of GHG with a protonated and deprotonated imidazole group. The pK-value was derived from the dissociation constant *K* by the calculation $pK = -\log(K)$. A pK value of 6.48 ± 0.08 was obtained from the fit.

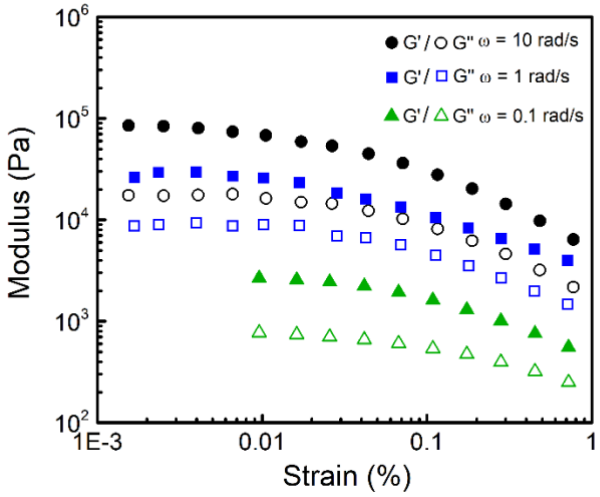

Figure S5. Amplitude dependence of the storage and loss moduli of the gel at three angular frequencies.

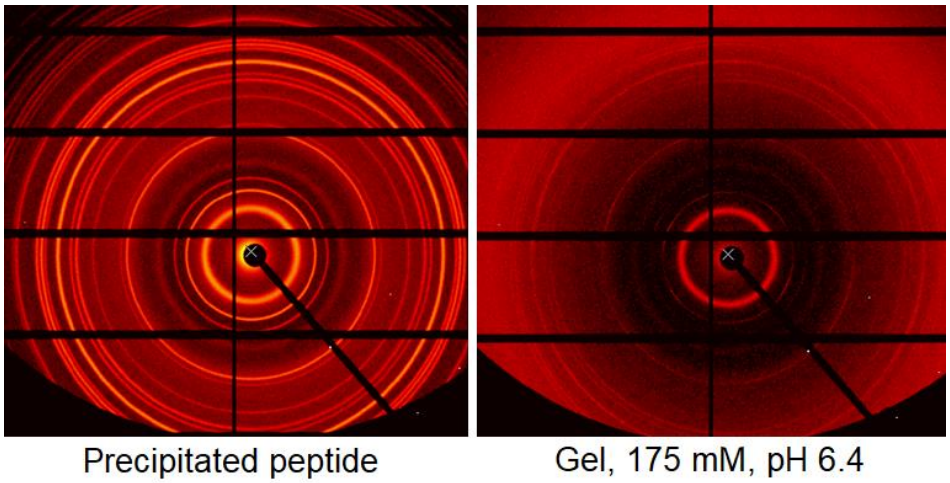

Figure S6. Wide-angle X-ray scattering. 2D patterns of precipitated peptide and gel with 175 mM GHG, pH=6.47.

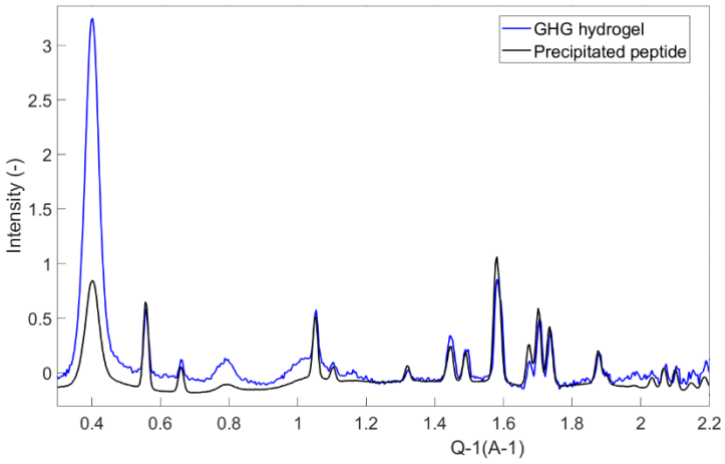

Figure S7. Wide-angle X-ray scattering of the precipitated peptide (black) and gel (blue) with 175 mM GHG, pH=6.47. The 2D patterns were integrated over a 360° angle. The amorphous component of the gel profile was subtracted.

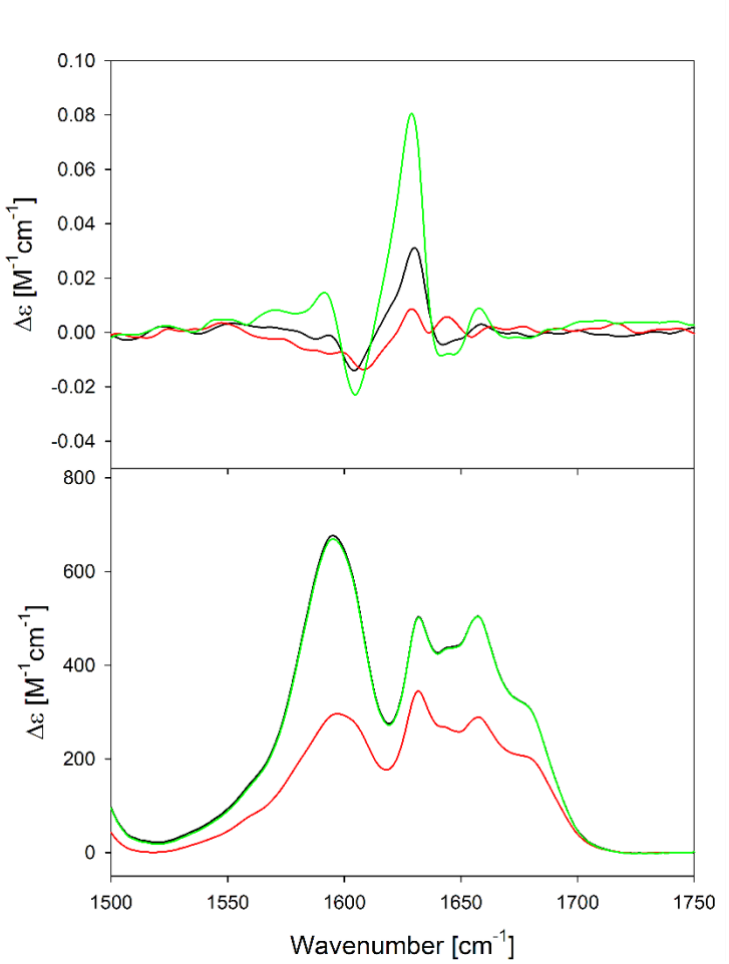

Figure S8. VCD (top) and FT-IR (bottom) spectra of 175 mM GHG at pD=6.39 in the region between 1500 and 1750 cm-1. Sample was rotated approximately 120° between scans. The red spectrum was scaled to the green spectrum at 1595 cm^{-1} for use in Figure 4 .

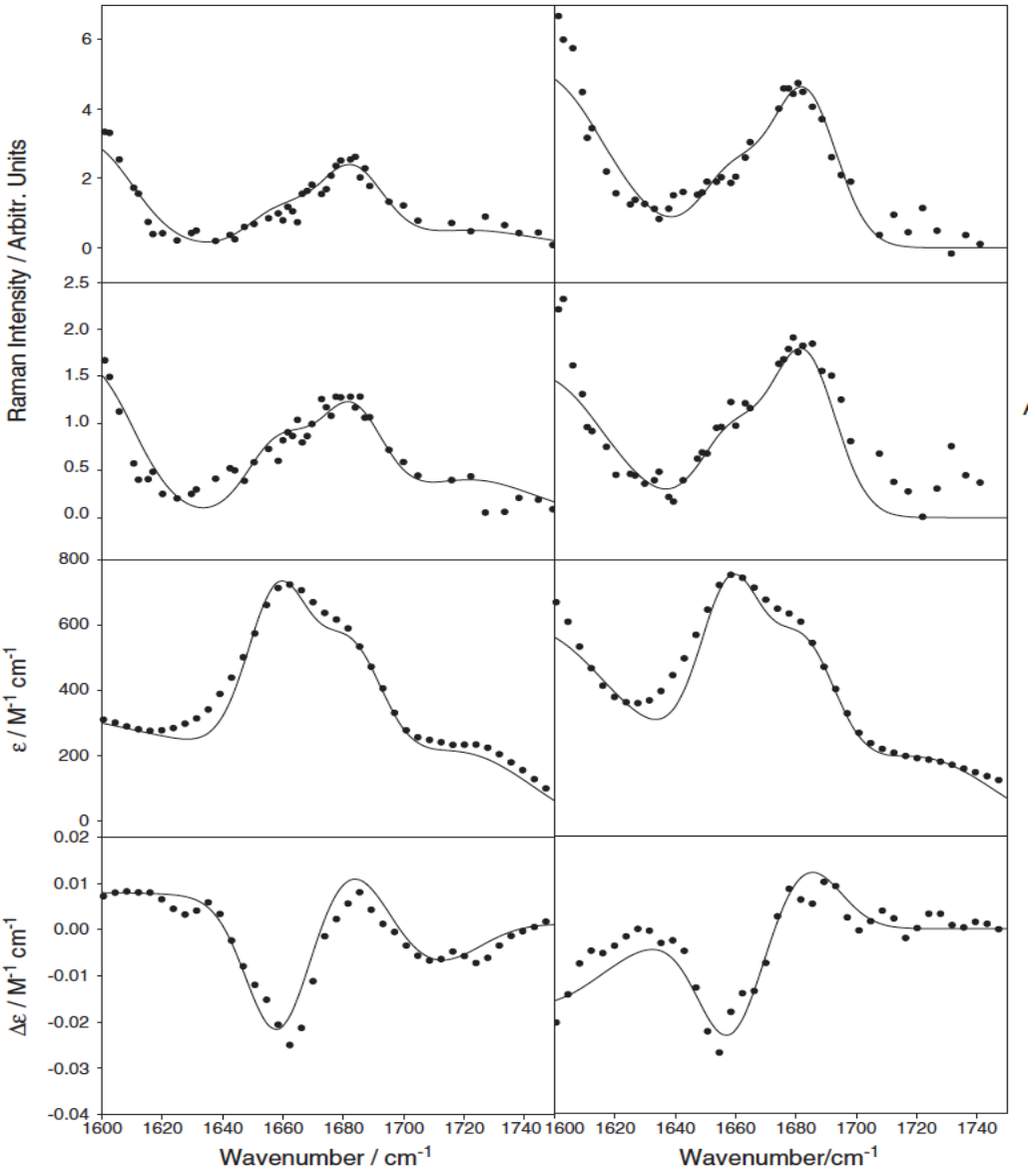

Figure S9. Isotropic Raman (top row), anisotropic Raman (second row), FTIR (third row) and VCD (fourth row) spectra of 200 mM double protonated GHG (left, imidazole and C-terminal protonated) and single protonated GHG (right, imidazole protonated, C-terminal deprotonated). The solid lines were the result of a fitting procedure described in the paper of DiGuiseppi et al. from which this figure was taken and modified.[1]