Supporting Information

Collagen-Inspired Helical Peptide Co-Assembly Forms a Rigid Hydrogel with Twisted Polyproline II Architecture

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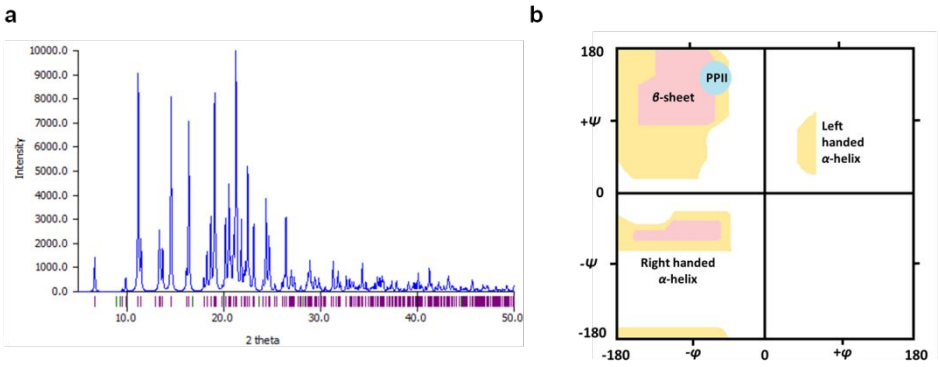

Figure S1: PXRD and Ramachandran Plot Position of the Fmoc-Gly-Pro-Hyp Crystal. (a) Single crystal XRD of an Fmoc-Gly-Pro-Hyp crystal obtained in 2:1 MeOH/water solvent mixture. (b) Ramachandran plot of the sterically allowed dihedral angles in the Polyproline II helical conformation (Blue region), which match the dihedral angle found in our Fmoc-Gly-Pro-Hyp crystal single unit.

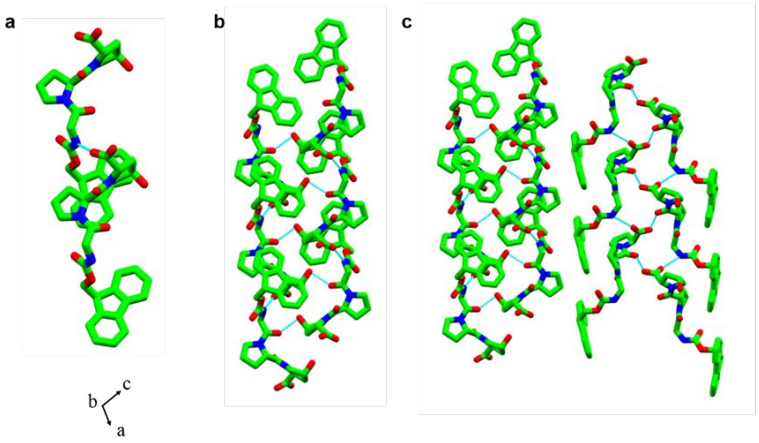

Figure S2: H-bonding sites of Fmoc-Gly-Pro-Hyp crystal. Side-by-side H-bond interactions of a single helical chain with two nearby chains in the crystal structure of Fmoc-Gly-Pro-Hyp, showing the H-bonding sites in the crystal structure.

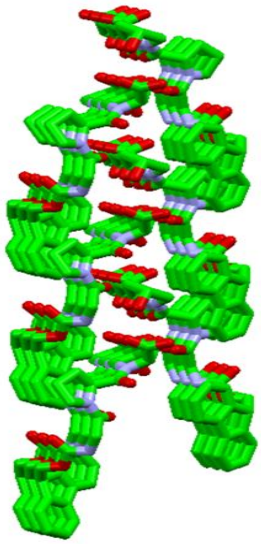

Figure S3: Trimeric Units of the Crystal Structure of Fmoc-Gly-Pro-Hyp. Interaction of trimeric units through H-bonds to produce the helical sheets in the crystal structure of Fmoc-Gly-Pro-Hyp.

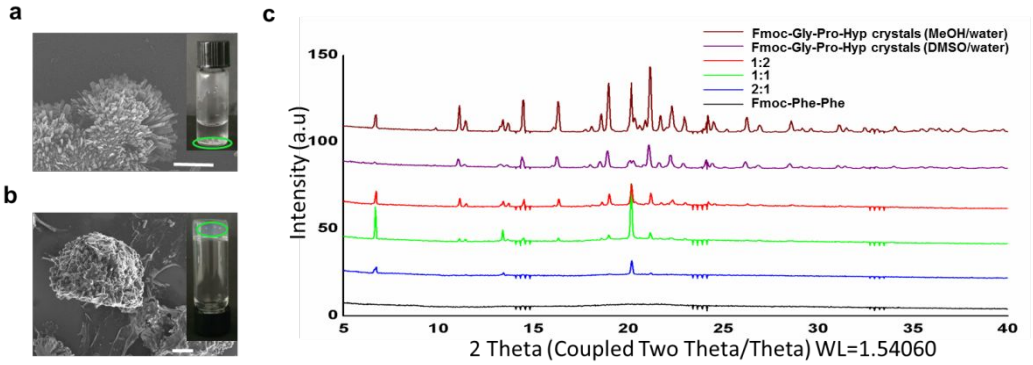

Figure S4: PXRD characterization of Co-assembly of Fmoc-Phe-Phe and Fmoc-Gly-Pro-Hyp. (a-b) SEM images of (a) Fmoc-Gly-Pro-Hyp crystals in 5% DMSO in water solvent, (b) Crystals after 5 days in the Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:2 hybrid hydrogel sample. Scale bar: 10 µm (c) PXRD characterization of dried Fmoc-Gly-Pro-Hyp in MeOH/water, Fmoc-Gly-Pro-Hyp in DMSO/water, Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 2:1, Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:1, Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:2 and Fmoc-Phe-Phe hydrogel.

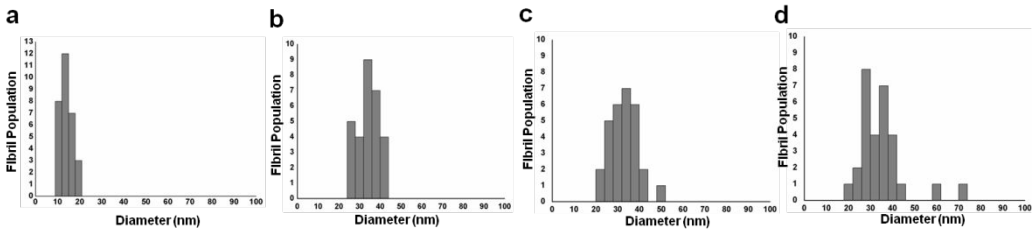

Figure S5: Hydrogels fibrils diameter determination by histogram plots from AFM image analysis. (a) FmocFF hydrogel, (b) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 2:1. (c) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:1. (d) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:2.

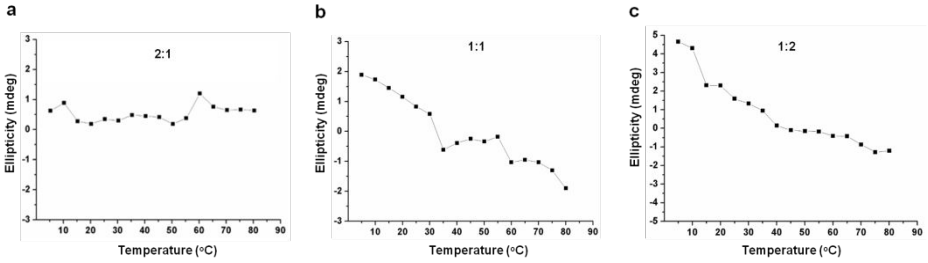

Figure S6: **Thermal Transition of Circular Dichroism Analysis of the 227 nm Positive Peak over a Temperature Scan of 5-90 °C**. (a) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 2:1.Circular dichroism analysis over a temperature scan of 5-90°C displaying the decrease in the positive peak intensity at 227 nm of (b) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:1, and (c) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:2.

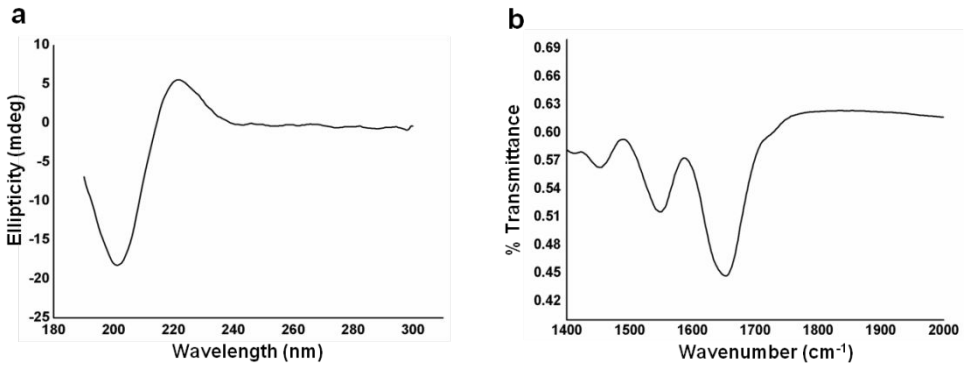

Figure S7: CD and FTIR Spectrum of Collagen. (a) CD spectrum of the Collagen. (b) FTIR spectrum of the Collagen.

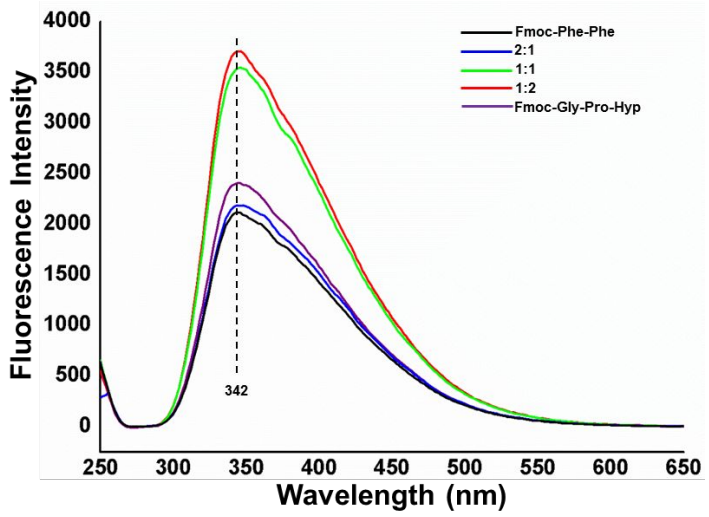

Figure S8: Fluorescence emission spectra. Fluorescence emission spectra at 342 nm of the fluorenyl moiety of the multi-component hydrogels of Fmoc-Phe-Phe and Fmoc-Gly-Pro-Hyp after excitation at 280 nm at time point 0 (initiation of gelations).

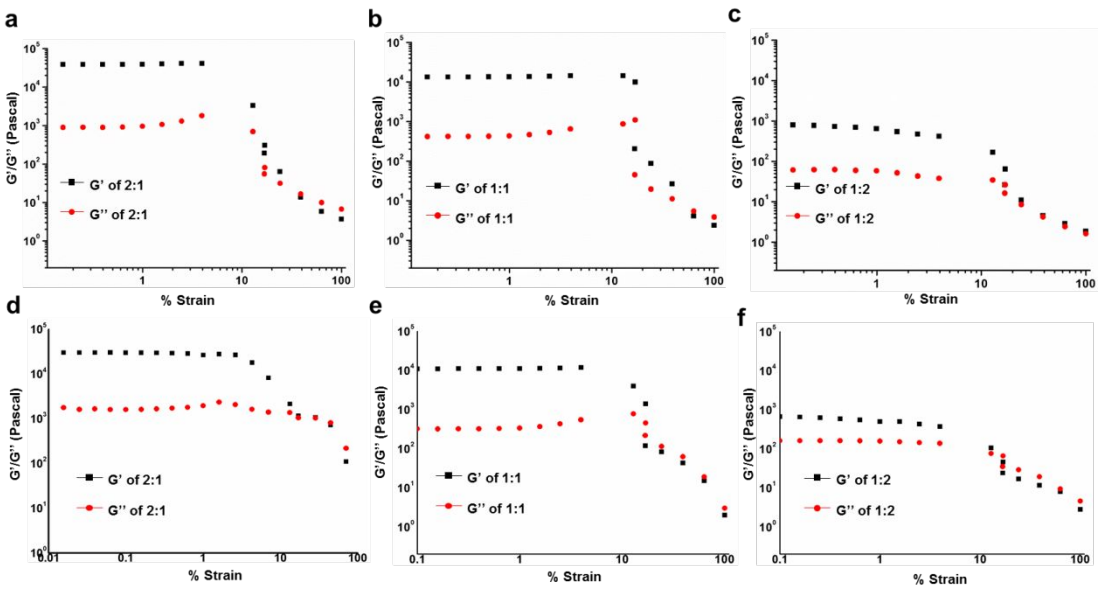

Figure S9: Rheological Strain Sweep Analyses of the Hybrid Hydrogels. Rheological strain sweep analyses showing the storage modulus (G') and loss modulus (G''). (a, d) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 2:1, (b, e) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:1, and (c, f) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:2 hybrid hydrogels.

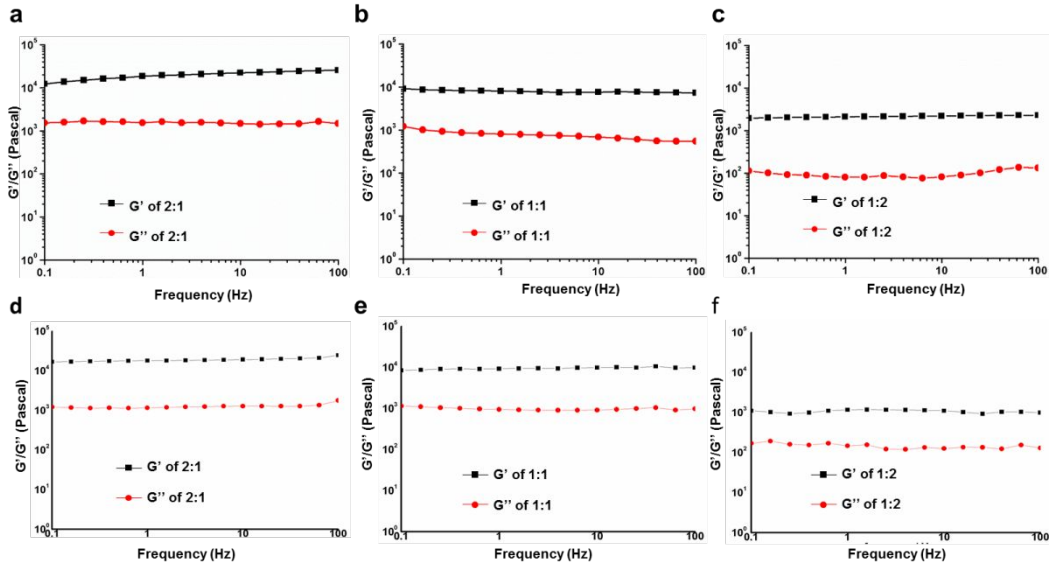

Figure S10: Rheological Frequency Sweep Analyses of the Hybrid Hydrogels. Rheological frequency sweep analyses showing the storage modulus (G') and loss modulus (G''). (a, d) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 2:1, (b, e) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:1 and (c, f) Fmoc-Phe-Phe:Fmoc-Gly-Pro-Hyp 1:2 hybrid hydrogels.

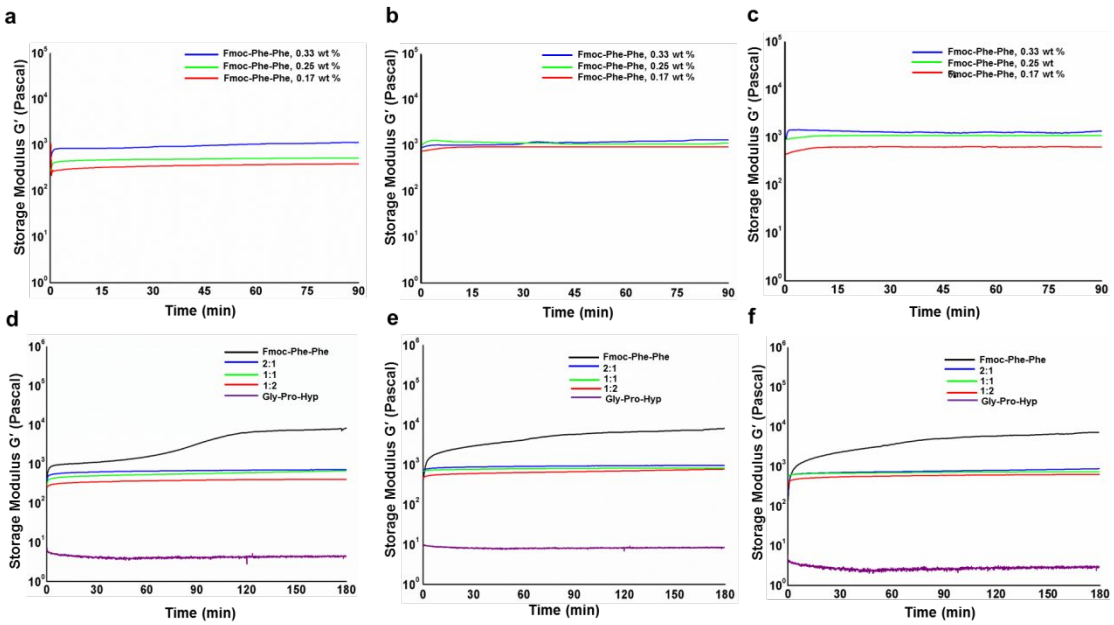

Figure S11: Rheological Time Sweep Analyses of Pure Fmoc-Phe-Phe and The Hybrid Hydrogels of Fmoc-Phe-Phe And Gly-Pro-Hyp. *In situ* time sweep oscillation measurements of hydrogel formation (a, b, c) Hydrogels formed by pure Fmoc-Phe-Phe at different concentrations (0.33, 0.25, 0.17 wt %). (d, e, f) Hydrogels formed by Fmoc-Phe-Phe, Fmoc-Phe-Phe:Gly-Pro-Hyp 2:1, Fmoc-Phe-Phe:Gly-Pro-Hyp 1:1, Fmoc-Phe-Phe:Gly-Pro-Hyp 1:2, and Gly-Pro-Hyp.

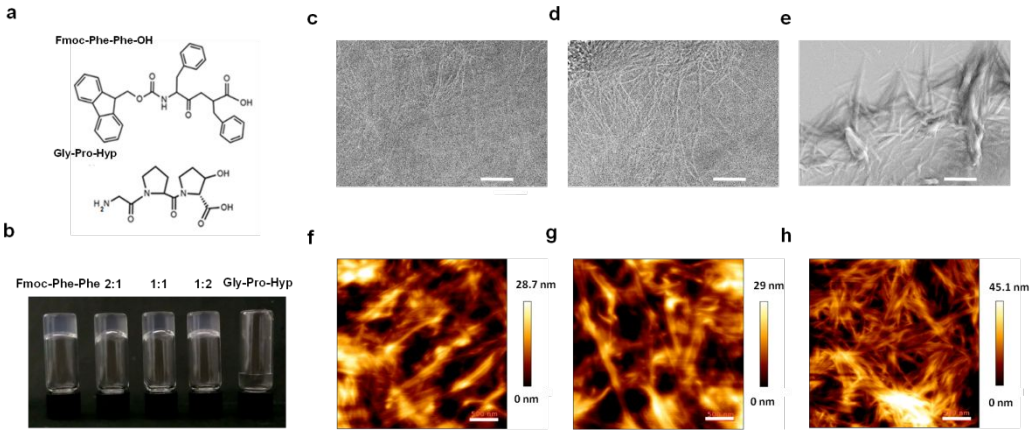

Figure S12: Co-Assembled Hydrogel of Fmoc-Phe-Phe and Gly-Pro-Hyp. (a) Molecular structure of the two building blocks, Fmoc-Phe-Phe and Gly-Pro-Hyp. (b) Inverted vials of the single and hybrid hydrogels. (c-e) SEM images of the studied hydrogels. Scale bar: 10 µm. (f-h) AFM images of the studied hybrid hydrogels. Scale bar: 500 nm. (c, f) Fmoc-Phe-Phe:Gly-Pro-Hyp 2:1. (d, g) Fmoc-Phe-Phe:Gly-Pro-Hyp 1:1. (e, h) Fmoc-Phe-Phe:Gly-Pro-Hyp 1:2.

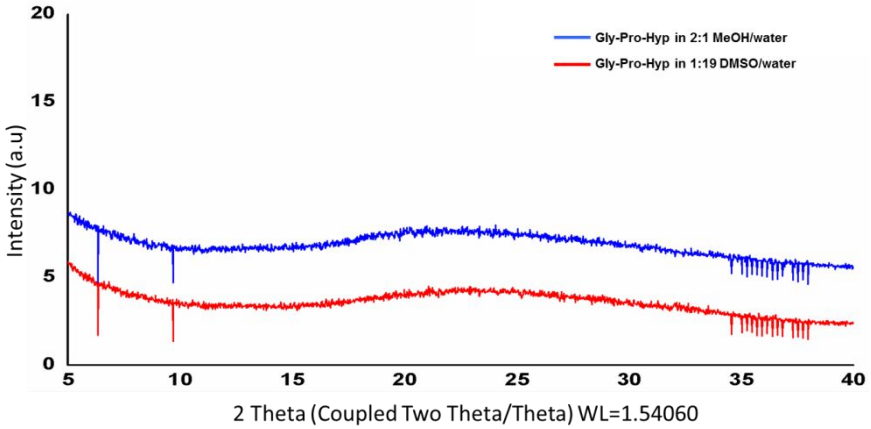

Figure S13: PXRD Characterization of Dried Gly-Pro-Hyp. PXRD characterization of dried Gly-Pro-Hyp in 2:1 MeOH/water and 1:19 DMSO/water solvent as indicated, showing no crystalline peak.

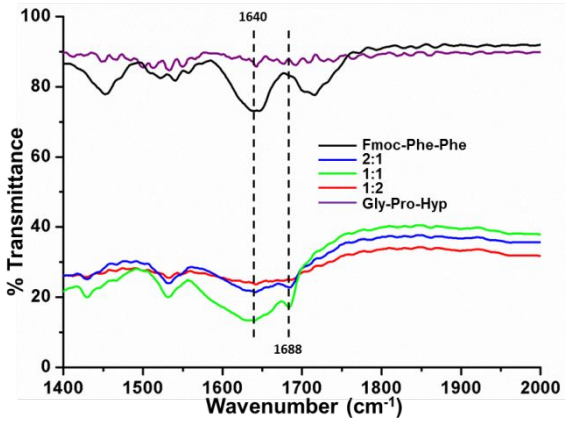

Figure S14: FTIR Spectra of the Hybrid Hydrogels Formed by Fmoc-Phe-Phe and Gly-Pro-Hyp. FTIR spectra of the hydrogels formed by Fmoc-Phe-Phe, Fmoc-Phe-Phe:Gly-Pro-Hyp 2:1, Fmoc-Phe-Phe:Gly-Pro-Hyp 1:1, Fmoc-Phe-Phe:Gly-Pro-Hyp 1:2, and Gly-Pro-Hyp displaying a β –sheet rich structure.

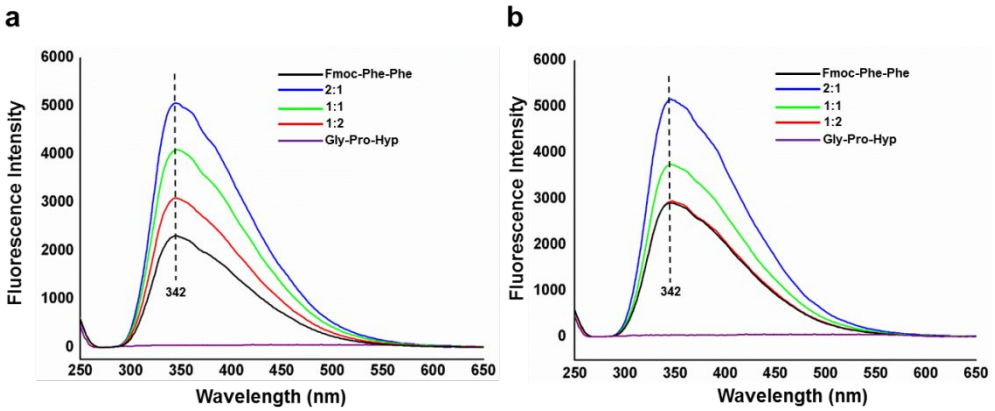

Figure S15: Fluorescence Emission Spectra. Fluorescence emission spectra at 342 nm of the fluorenyl moiety of the multi-component hydrogels of Fmoc-Phe-Phe and Gly-Pro-Hyp under excitation of 280 nm at (a) time point 0 and (b) 3 hours after gel formation showing no peak shift.

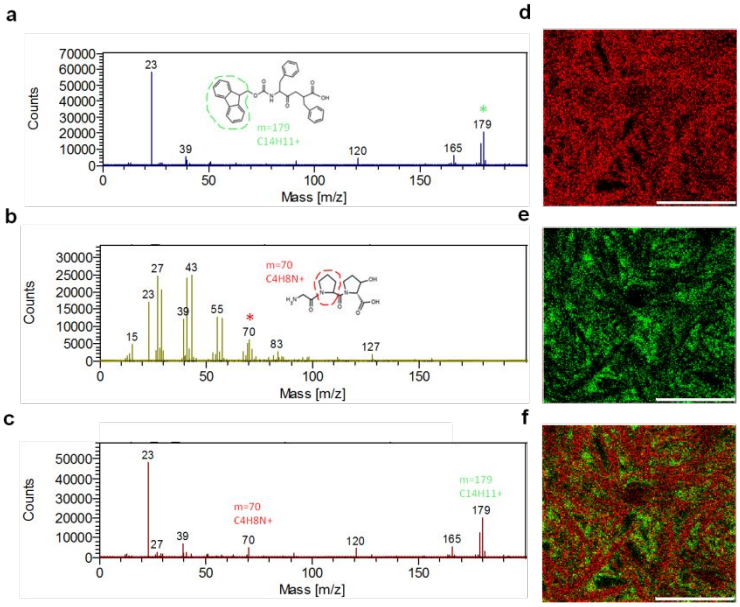

Figure S16: ToF-SIMS Analysis of the Chemical Composition of the Fmoc-Phe-Phe:Gly-Pro-Hyp 1:1 Hybrid Hydrogel. (a-c) ToF-SIMS Mass spectrometry analysis. (d-f) Chemical ion maps. (a, d) Fmoc-Phe-Phe, (b, e) Gly-Pro-Hyp, (c, f) Fmoc-Phe-Phe:Gly-Pro-Hyp 1:1 hybrid hydrogel. Figure f represents a merged image of d and e. Scale bar represents 10 μm.

Complex	**Fmoc-Gly-Pro-Hyp**
CCDC Deposition #	1962894
Formula	C_{27} H_{29} N_3 O_7
Crystal description	colorless tablet
Crystal size, [mm^3]	0.03 x 0.073 x 0.161
FW, [$g.mol^{-1}$]	507.53
Space group	$P2_12_12_1$
Crystal system	Orthorhombic
a, [Å]	9.4887(1)
b, [Å]	9.8639(1)
c, [Å]	26.3497(2)
α, [°]	90
β, [°]	90
γ, [°]	90
Cell volume, [$Å^3$]	2466.22(4)
Z	4
ρ_{cacld}, [$g.cm^{-3}$]	1.367
μ, [mm^{-1}]	0.826
No. of reflections	27983
No. of unique reflections	5348
$2\Theta_{max}$, [°]; completeness %	67.68; 99.9
R_{int}	0.0347
No. of parameters (restraints)	336(0)
Final R[a], wR2	0.0308, 0.0769
Final R[b], wR2	0.0313, 0.0773
GooF	1.043

Table S1. Crystallographic data of Fmoc-Gly-Pro-Hyp.

[a] for data with $I > 2\sigma(I)$. [b] for all data.