Supporting information

Co-assembled supramolecular hydrogels: Nano-IR sheds light on tripeptide assemblies

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Table of Contents

S1. Spectroscopic data for D-His-L-Phe-L-Phe-NH_2 (hFF)

The spectroscopic data for this tripeptide corresponded to the literature, as published in M. Kurbasic, Ana M. Garcia, S. Viada, and S. Marchesan, *Molecules* **2020**, *26*, 173: ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.89 (s, 1H, NH), 8.68 (d, *J* = 8.7 Hz, 1H, NH), 8.47 (d, *J* = 8.2 Hz, 1H, NH), 8.15 (s, 3H, NH_3^+), 7.44 (s, 1H, Ar), 7.32 – 7.03 (m, 10H, Ar), 6.89 (s, 1H, Ar), 4.76 – 4.64 (m, 1H, αCH), 4.46 (td, *J* = 8.7, 5.1 Hz, 1H, αCH), 4.15 – 4.01 (m, 1H, αCH), 3.06 (dd, *J* = 3.9 Hz, J_{gem} = 13.8 Hz, 1H, βCH_2), 3.01 (dd, *J* = 5.1 Hz, J_{gem} = 13.8 Hz, 1H, βCH_2), 2.90 (dd, *J* = 4.7 Hz, J_{gem} = 15.8 Hz, 1H, βCH_2), 2.82 (dd, *J* = 9.2 Hz, J_{gem} = 13.9 Hz, 1H, βCH_2), 2.72-2.63 (m, 2H, βCH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 173.1, 170.9, 167.3 (3 x CO); 138.3, 137.7, 134.8, 129.7, 129.6, 128.6, 128.4, 126.8, 119.0, 117.3, 115.9 (Ar); 54.6, 54.1, 51.6 (3 x αC); 38.7, 38.2, 26.8 (3 x βC). ESI-MS m/z 449.2 $(M+H)^+$ $C_{24}H_{28}N_6O_3$ requires 449.2.

S2. Spectroscopic data for L-His-D-Phe-D-Phe-NH_2 (Hff)

The spectroscopic data for this tripeptide corresponded to the literature, as published in Mari C. Manas Torres, P. Alletto, S. Adorinni, A. V. Vargiu, L. Alvarez Cienfuegos, and S. Marchesan, *Org. Biomol. Chem.* **2025**, *doi: 10.1039/D4OB01987C*. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.79 (s, 1H, NH), 8.69 (d, *J* = 8.9 Hz, 1H), 8.48 (d, *J* = 8.4 Hz, 1H), 8.15 (s, 2H, NH_2), 7.46-6.88 (m, 14H, Ar, $CONH_2$), 4.70 (td, *J* = 10.1, 4.0 Hz, 1H, αCH), 4.47 (td, *J* = 8.9, 5.2 Hz, 1H, αCH), 4.07 (dd, *J* = 9.2, 3.5 Hz, 1H, αCH), 3.10 – 3.00 (m, 2H, βCH_2), 2.92 – 2.80 (dd, *J* = 13.9, 9.2 Hz, 2H, βCH_2), 2.72 – 2.63 (m, 2H, βCH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 173.1, 170.9, 167.3 (3 x CO); 138.3, 137.7, 134.8, 129.7, 129.6, 128.6, 128.4, 126.8, 119.0, 117.3, 115.9 (Ar); 54.6, 54.1, 51.6 (3 x αC); 38.7, 38.2, 26.8 (3 x βC). ESI-MS m/z 448.6 $(M+H)^+$ $C_{24}H_{28}N_6O_3$ requires 449.2.

S3. Spectroscopic data for L-Asp-D-Phe-D-Phe-NH_2 (Dff)

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.93 (s (br), 1H, COOH), 8.58 (d, *J* = 8.8 Hz, 1, NH), 8.40 (d, *J* = 4.4 Hz, 1, NH), 7.99 (s(br), 3, NH_3^+), 7.45 (s, 1, NH_2), 7.31-7.16 (m, 10, Ar), 7.10 (s, 1, NH_2), 4.67 (ddd, *J* =4 Hz, *J* = 8.8 Hz, 10.4 Hz, 1, CHα), 4.47 (ddd, *J* = 5.2 Hz, *J* = 8.4 Hz, *J* = 8.8 Hz, 1, CHα), 3.96 (b, 1, CHα), 3.07 (dd, *J* = 4 Hz, *J* = 14 Hz, 1H, CH_β), 3.02 (dd, *J* = 5.2 Hz, *J* = 14 Hz, 1, CH_β),

2.82 (dd, *J* = 8.8 Hz, *J* = 14 Hz, 1, CH_β), 2.65 (dd, *J* = 10.4 Hz, *J* = 14 Hz, 1, CH_β), 2.39 (dd, *J* = 3.6 Hz, *J* = 17.6 Hz, 1, CH_β), 2.27 (dd, *J* = 9.2 Hz, *J* = 18.1 Hz, CH_β). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm) 172.6, 170.9, 170.4, 167.13 (4 x CO); 137.8, 137.2, 129.2, 129.2, 128.1, 127.9, 126.4, 126.3 (Ar); 54.02, 53.5, 48.7 (3 x αC); 39.2, 37.7, 35.7 (3 x βC). ESI-MS m/z 424.6 $(M-H)^-$ $C_{22}H_{25}N_4O_5$ requires 425.2.

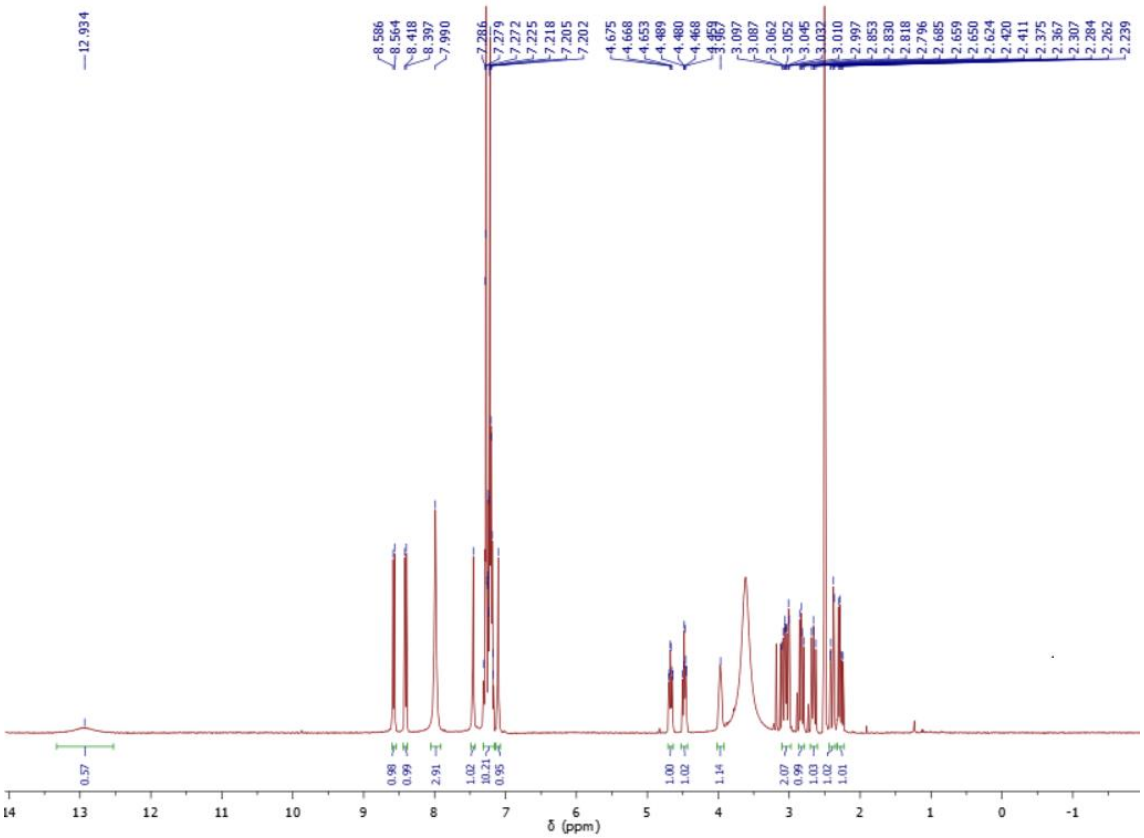

Figure S1. ^{1}H-NMR spectrum of L-Asp-D-Phe-D-Phe-NH_2 (Dff).

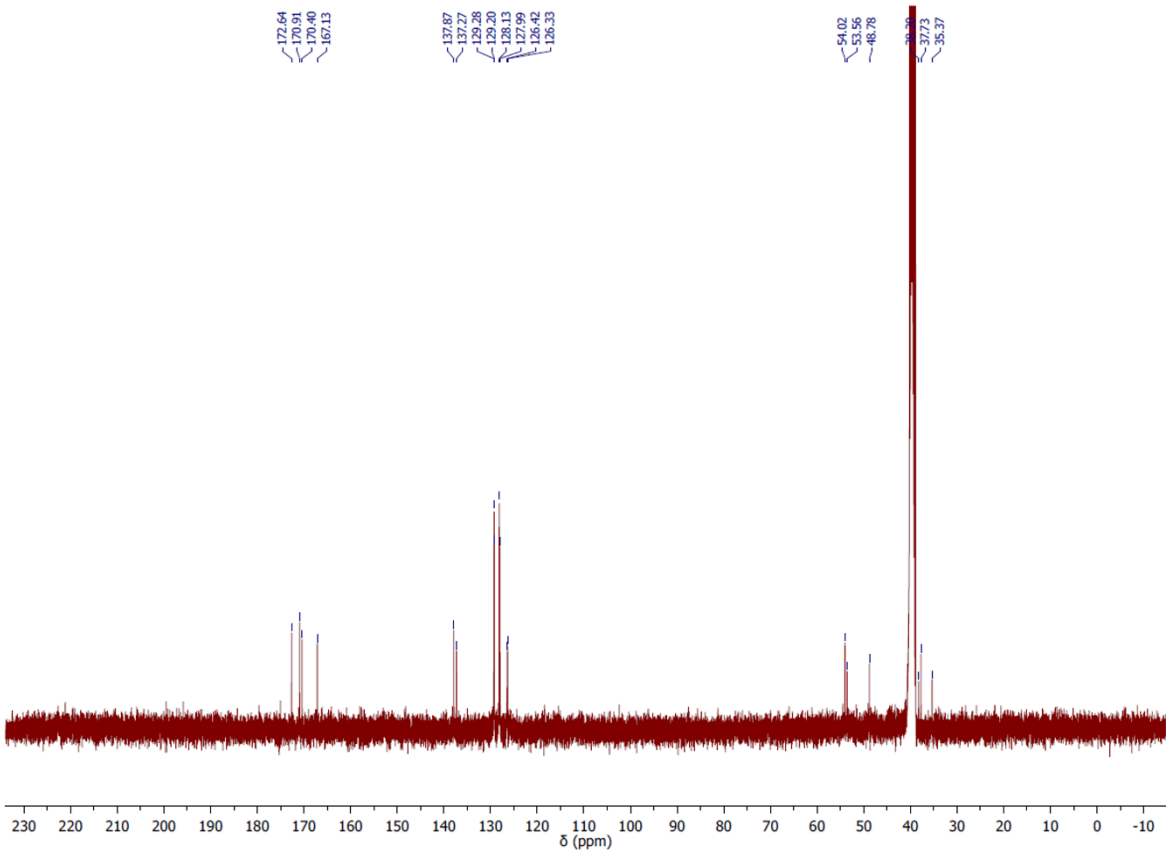

Figure S2. ^{13}C-NMR spectrum of L-Asp-D-Phe-D-Phe-NH_2 (Dff).

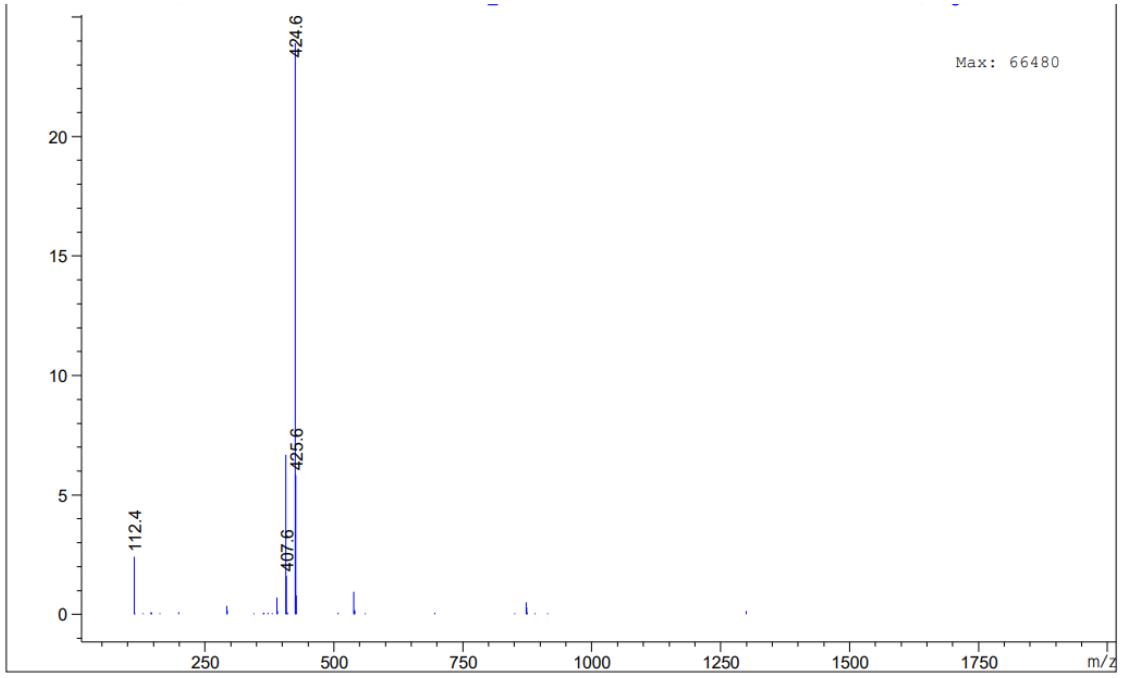

Figure S3. ESI-MS spectrum (negative ion mode) of L-Asp-D-Phe-D-Phe-NH_2 (Dff).

S4. Oscillatory rheology

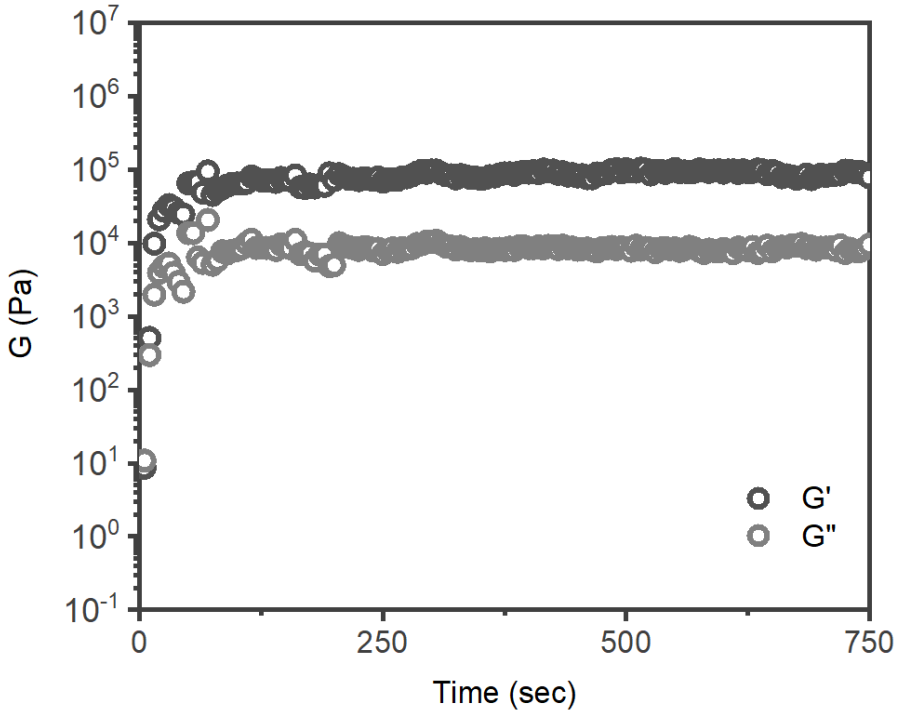

Figure S4. Time sweep of Dff hydrogel (25 mM).

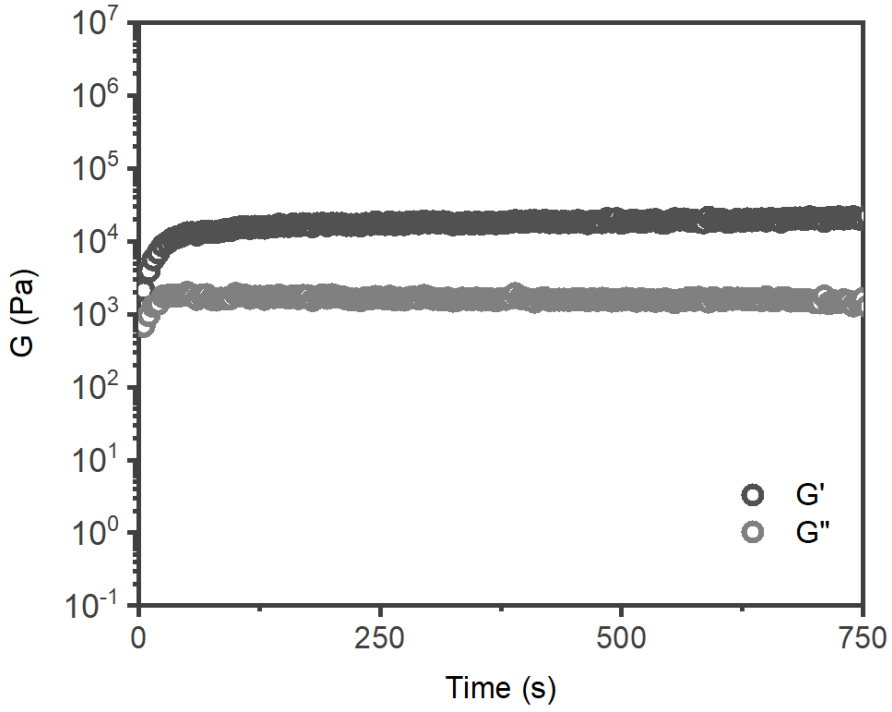

Figure S5. Time sweep of Hff/Dff co-assembled hydrogel (25 mM each).

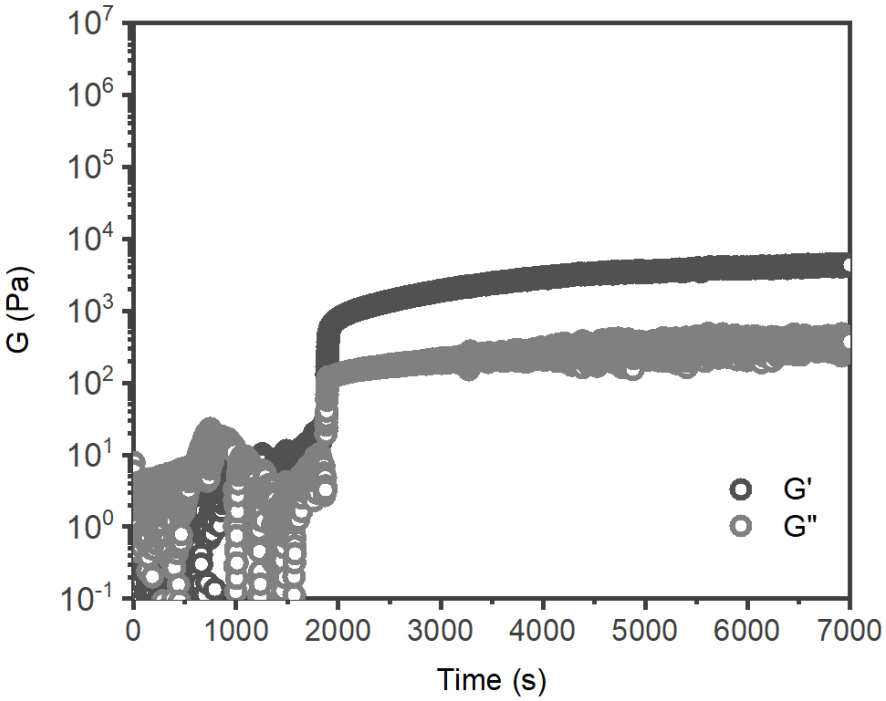

Figure S6. Time sweep of hFF/Dff co-assembled hydrogel (25 mM each).

S5. Thermoreversibility tests

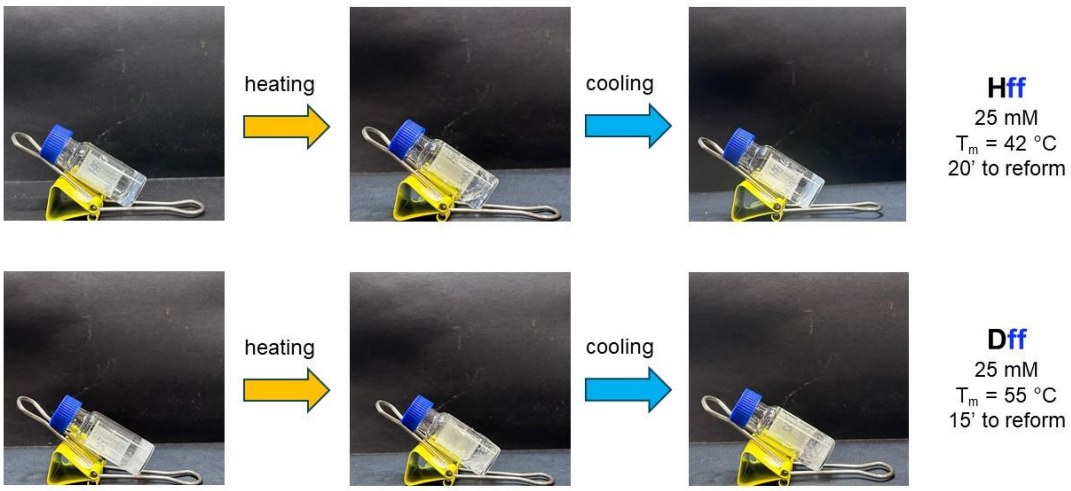

Figure S7. Thermoreverisbility test for Hff and Dff hydrogels at 25 mM.

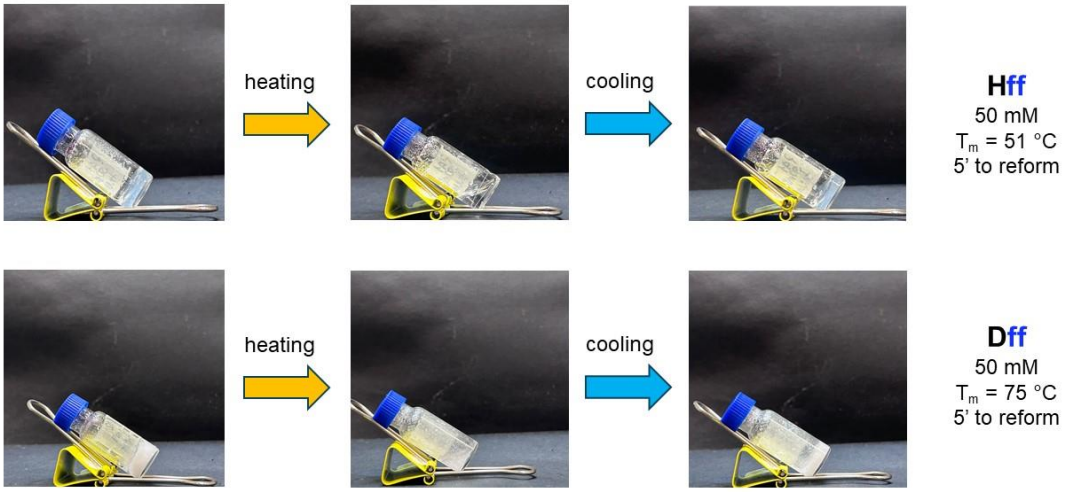

Figure S8. Thermoreverisbility test for Hff and Dff hydrogels at 50 mM.

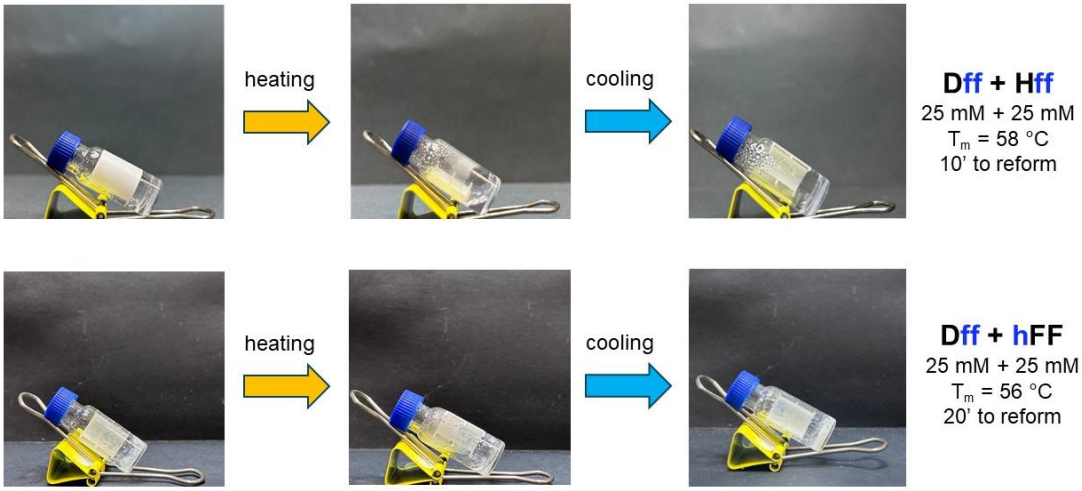

Figure S9. Thermoreverisbility test for multicomponent hydrogels.

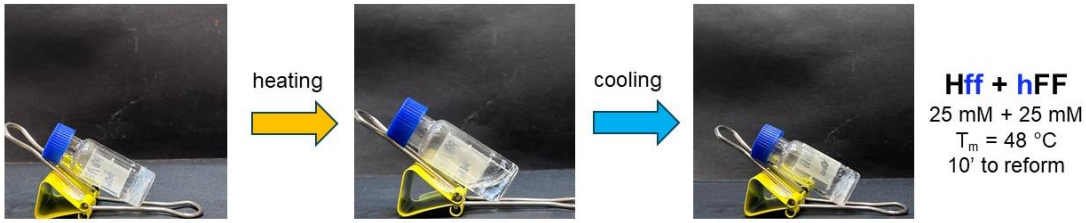

Figure S10. Thermoreverisbility test for racemic hydrogel.

S6. Atomic Force Microscope (AFM)

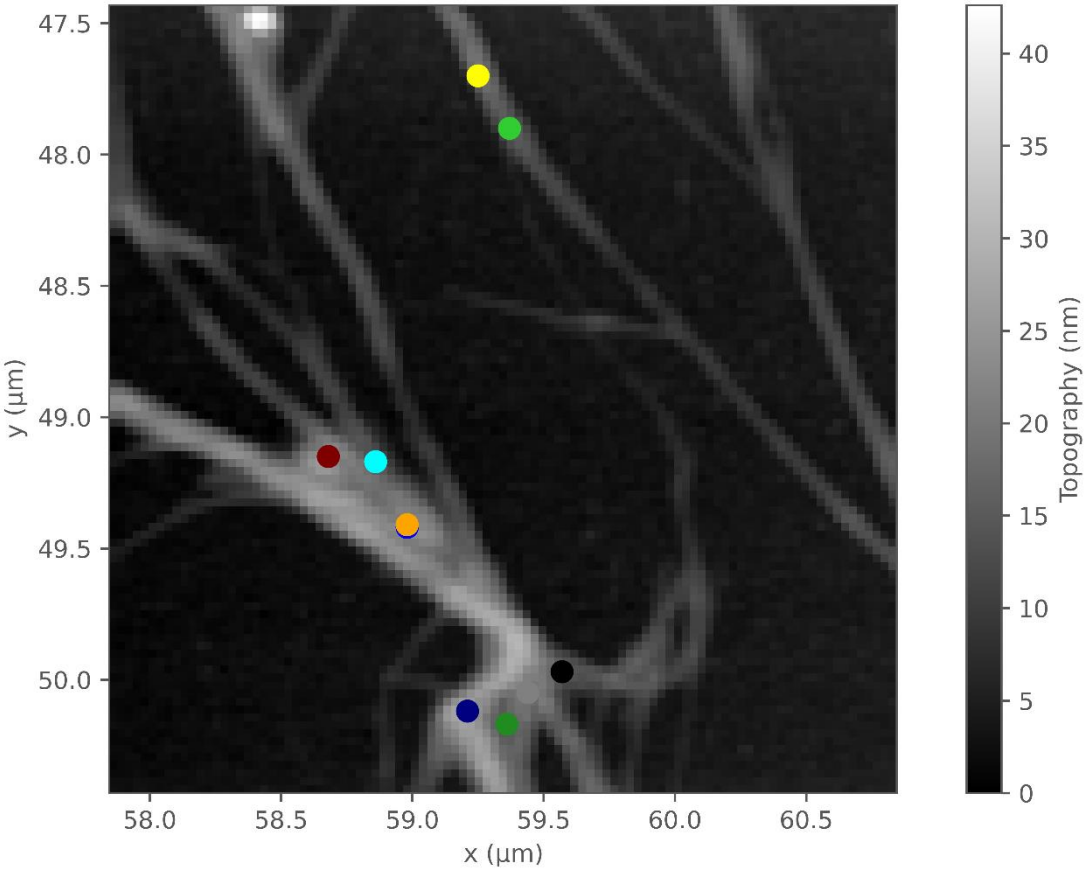

Figure S11. AFM image of Dff/Hff co-assembled gel (25 mM each). Colored points indicate the position of infrared spectra acquisition.

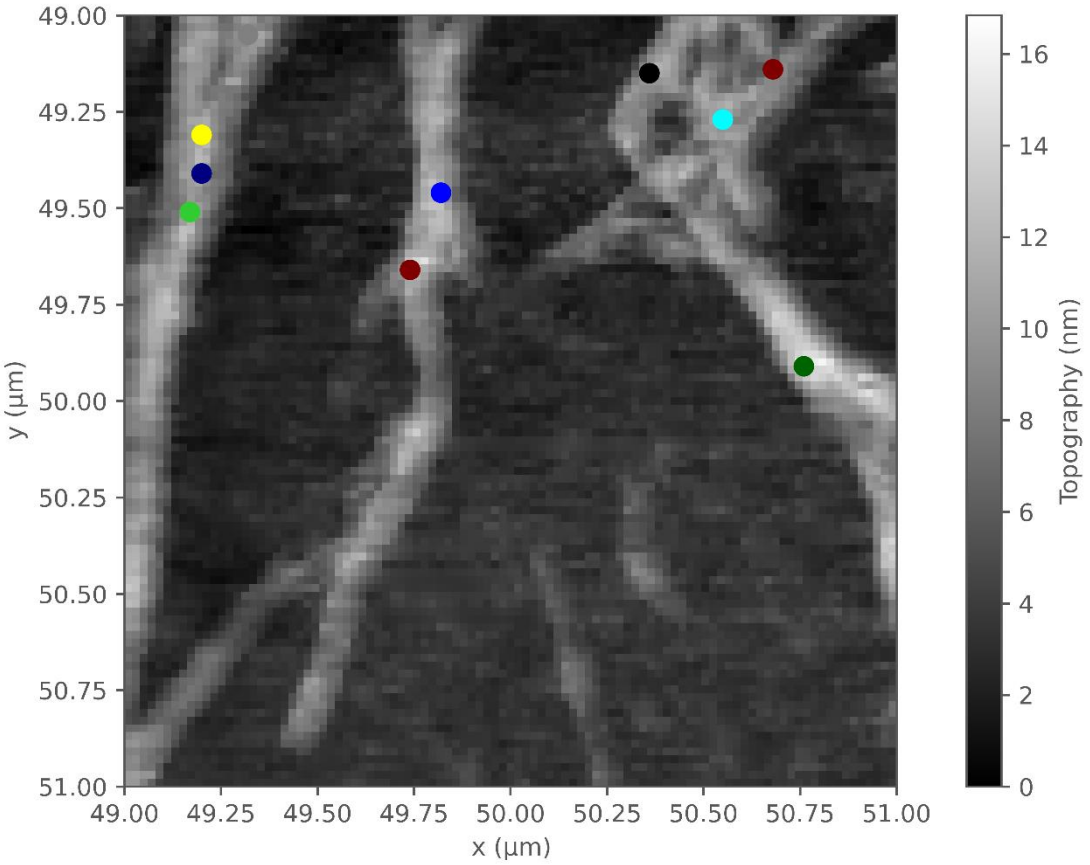

Figure S12. AFM image of Dff/hFF co-assembled gel (25 mM each). Colored points indicate the position of infrared spectra acquisition.

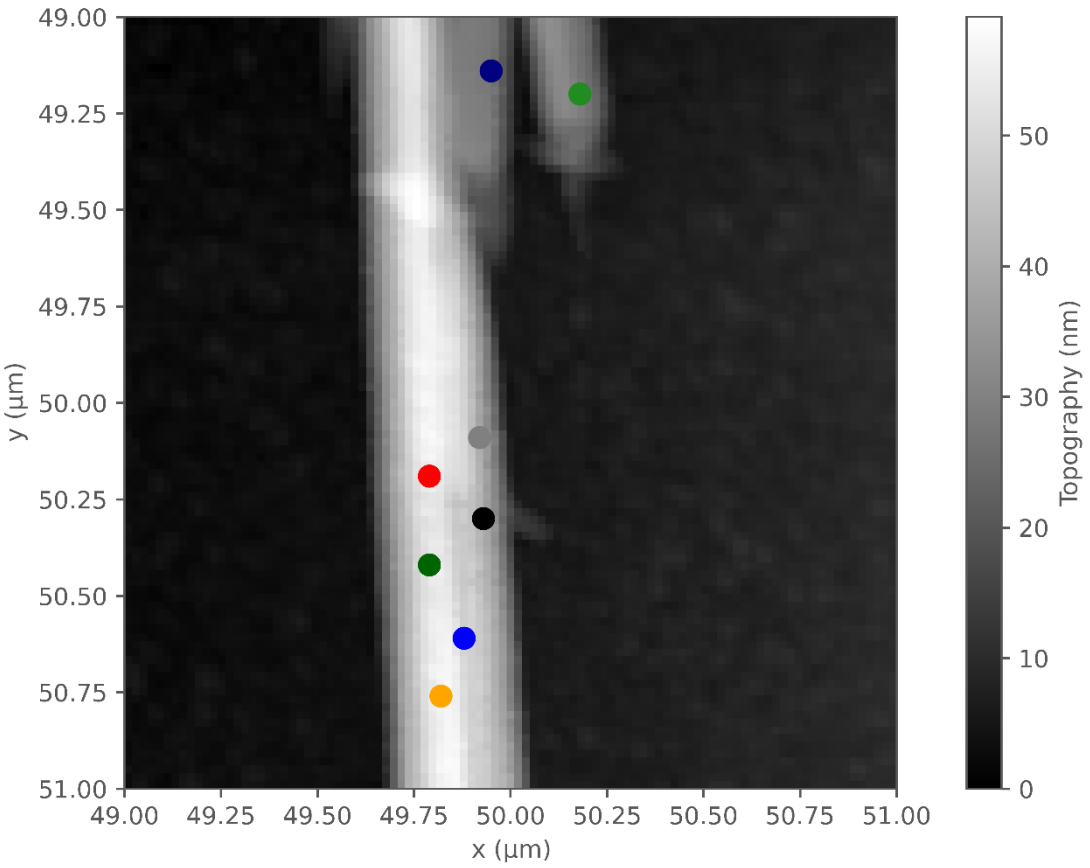

Figure S13. AFM image of hFF/Hff co-assembled gel (12.5 mM each). Colored points indicate the position of infrared spectra acquisition.

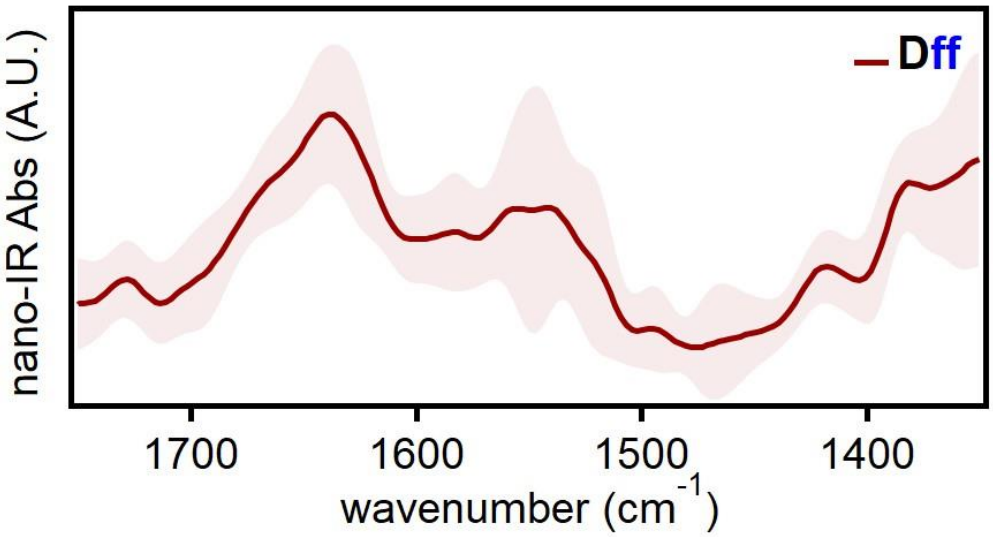

Figure S14. Nano-IR data average profile for Dff sample.

S7. ^{1}H-NMR spectra of multicomponent samples

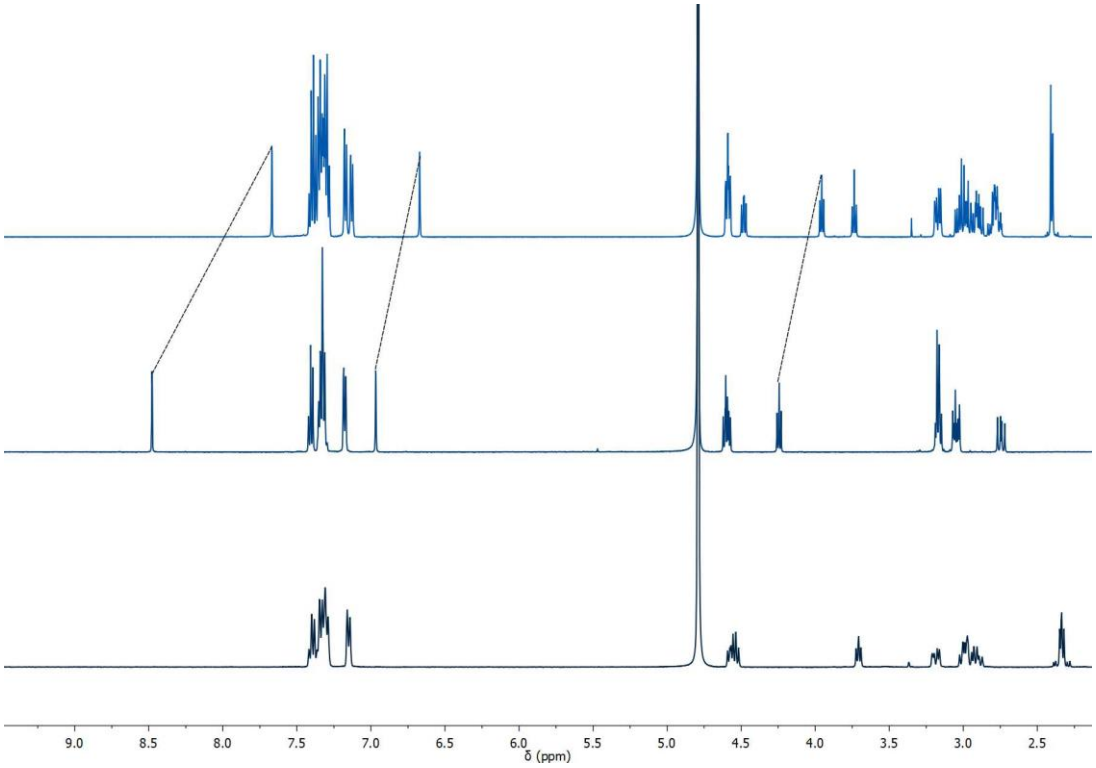

Figure S15. Stacked ^{1}H-NMR spectra of Hff+ Dff (top), Hff (middle), and Dff (bottom) in D_2O at pH 7. The most ignificant shifts are marked with dotted lines (His imidazole CHs and C_α-H).

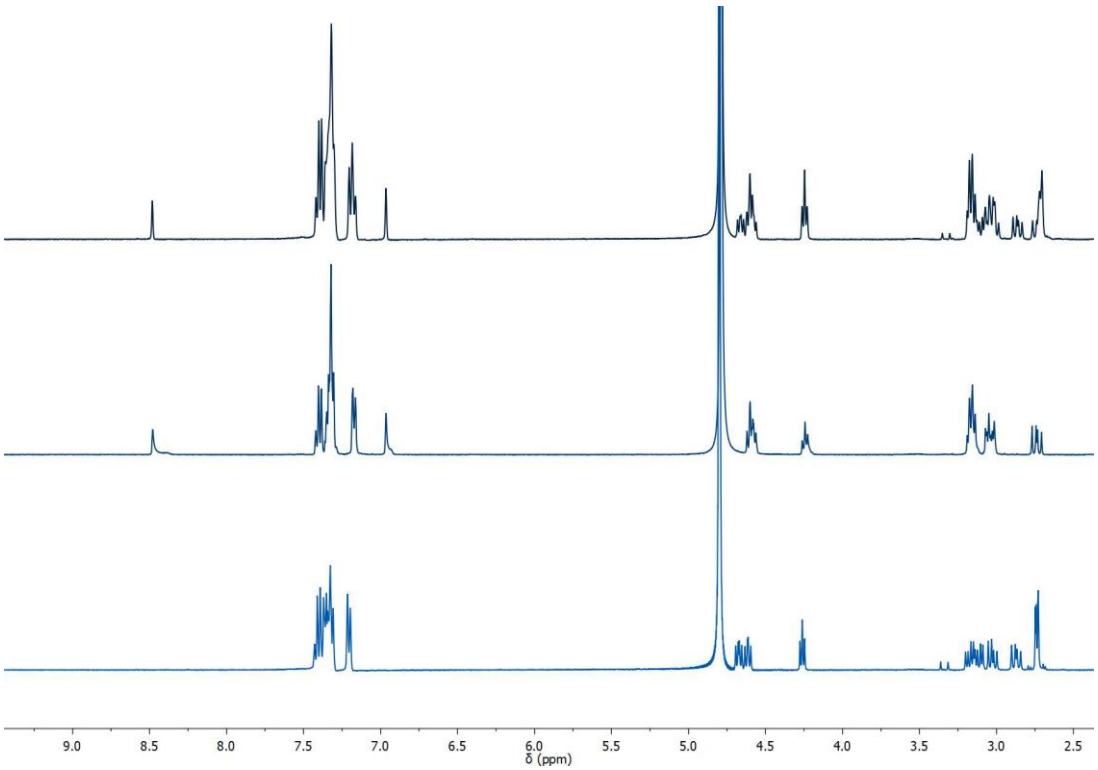

Figure S16. Stacked ^{1}H-NMR spectra of Hff+ Dff (top), Hff (middle), and Dff (bottom) in D_2O at pH 2. No significant shifts are present.

S8. Catalytic Data

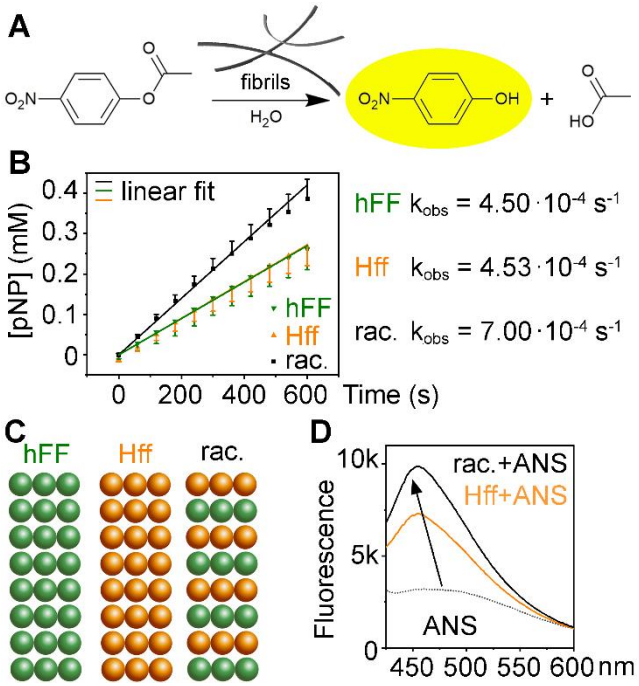

Figure S17. A) Reaction scheme for p-nitrophenyl acetate (pNPA) hydrolysis catalysed by peptide fibrils to yield yellow p-nitrophenol (pNP). **B)** Initial reaction rate in the presence of fibrils of hFF, Hff, or their racemic mixture (rac.). **C)** Scheme of the proposed assemblies of enantiopure stacks for hFF or Hff, and of alternating enantiomers for the racemic mixture. **D)** Fluorescence emission of ANS in Hff and racemic (rac.) peptide fibers, and in solution. Reproduced from M. C. Mañas-Torres, P. Alletto, S. Adorinni, A. V. Vargiu, L. Álvarez de Cienfuegos, and S. Marchesan, *Org. Biomol. Chem.* **2025**, *doi: 10.1039/D4OB01987C*.

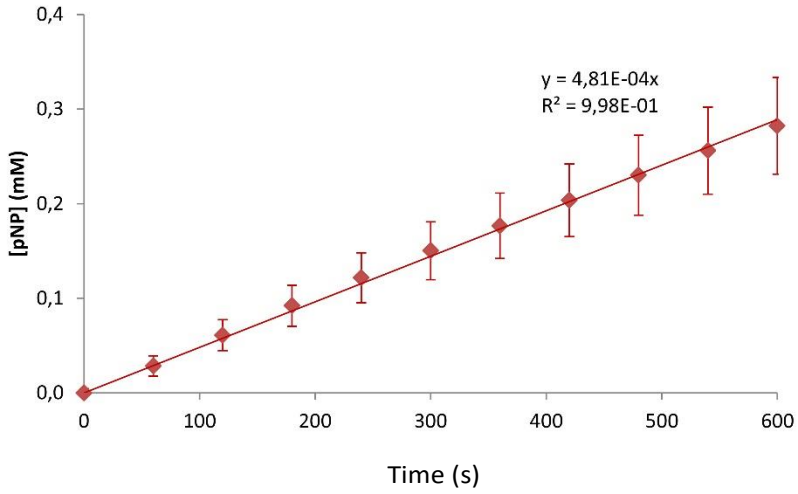

Figure S18. Initial reaction rate for samples composed of hFF and Dff, each one at 25 mM, with 1 mM pNPA.

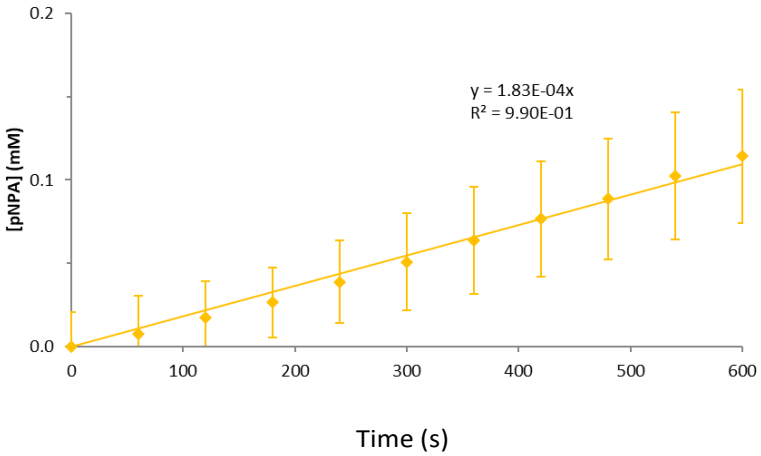

Figure S19. Initial reaction rate for samples composed of Hff and Dff, each one at 25 mM, with 1 mM pNPA.