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Rational Design of Poly(Peptide-Ester) Block Copolymers for Enzyme-Specific Surface Resorption

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Supplementary Data

Methods

Solubility of 1,4-phenylenediacetic acid

Solutions of 1,4-phenylenediacetic acid (PDA) were prepared at 0.1 mg/mL. Samples were also prepared above saturation in sterile PBS by adding enough solid such that particulates were still clearly visible after incubation. Vials were incubated at 37°C with shaking for 8 hours. Eight serial two-fold dilutions were prepared starting at 0.1 mg/mL to generate a standard curve. Saturated solution was filtered through a 0.22 μm PES filter, and then diluted 1:10 and 1:100 in sterile PBS. 10 μL injections were analyzed by analytical HPLC as described under General Procedures. Standard curve was used to calculate concentration of PDA in saturated solutions.

Peptide synthesis

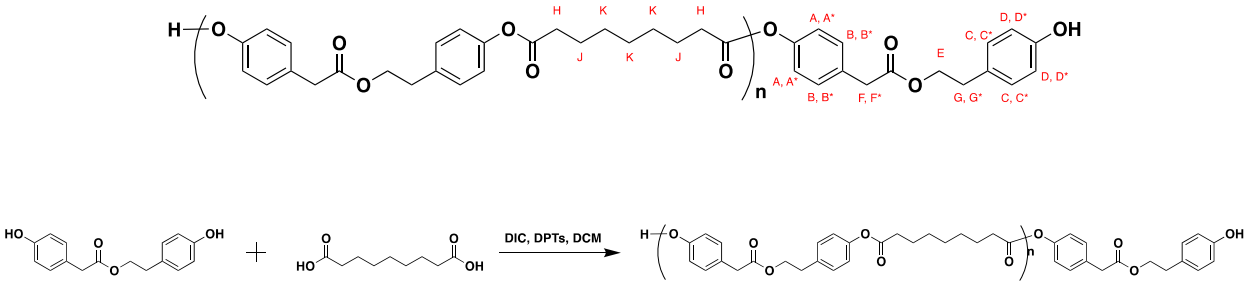
Amino acids were coupled sequentially to a Rink amide resin using HCTU. For each coupling step, the Fmoc-protected amino acid, HCTU, and diisopropylethylamine (DIPEA) was added to the growing resin at a ratio of (4:3.95:6) molar equivalents in DMF. A ninhydrin test (AnaSpec) was used to confirm completion of each coupling. The Fmoc protection group was removed through two resin washes with 20% piperidine in DMF for five minutes each. A ninhydrin test was performed again to confirm deprotection. After coupling the last glycine, the peptide was capped with trityl-protected mercaptoproprionic acid. The peptide was cleaved from the resin using a solution of 92.5% TFA, 2.5% dithiothreitol (DTT), 2.5% triisopropylsilane, and 2.5% water. The cleaved peptide solution was filtered from the solid resin and excess TFA was removed by rotary evaporation. The peptide was precipitated into a 10-fold excess of DEE and pelleted using centrifugation. The solid precipitate was washed twice with DEE, filtered, and

dried under vacuum. The crude peptide was dissolved in ultrapure water, and the pH of the peptide solution was adjusted to 6.5 using ammonium hydroxide. The purity of peptide was evaluated by analytical HPLC prior and after purification. Purification of the peptide was performed using flash chromatography (Teledyne Combiflash Rf) under acidic conditions using a 10-minute gradient flow from 10%/90% acetonitrile/water to 100% acetonitrile at 40 mL/min. Purified fractions were collected, excess acetonitrile was removed by rotary evaporation, and the remaining solution was frozen and lyophilized.

Synthetic Data and Schemes

Synthesis of poly(HTy azelate) (r = 0.8)

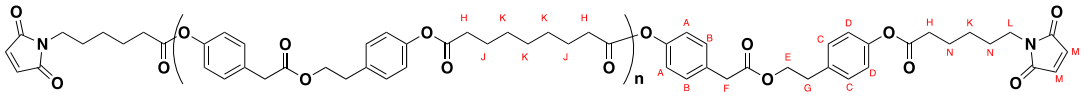
GPC (Chloroform + 0.1% TFA): M_n = 5.9 kDa, M_w = 9.0 kDa, PDI = 1.5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.24 (**A**, d, J = 8.4 Hz, 24H), 7.19 (**A***, d, J = 8.5 Hz, 5H), 7.15 (**B**, d, J = 8.4 Hz, 26H), 7.09 (**B***, d, J = 8.4 Hz, 3H), 7.03 (**C**, d, J = 8.6 Hz, 25H), 6.99 (**D**, d, J = 8.5 Hz, 29H), 6.95 (**C***, d, J = 8.5 Hz, 3H), 6.91 (**D***, d, J = 8.6 Hz, 4H), 6.71 (d, J = 8.5 Hz, 3H), 6.63 (d, J = 8.5 Hz, 4H), 4.28 (E, t, J = 7.0 Hz, 37H), 3.58 (F, s, 27H), 3.54 (F*, s, 5H), 3.50 (F*, s, 3H), 2.90 (G, t, J = 7.0 Hz, 32H), 2.82 (G*, t, J = 6.7 Hz, 5H), 2.56 (H, t, J = 7.5 Hz, 65H), 1.76 (J, q, J = 7.7, 7.2 Hz, 64H), 1.60 (s, 7H), 1.48 – 1.40 (K, m, 94H).

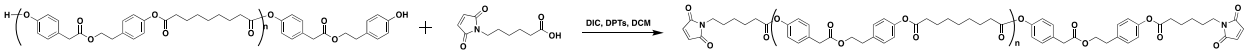


Scheme S1. Reaction schematic for synthesis of poly(HTy azelate).

Synthesis of poly(HTy azelate) (r = 0.8) bismaleimide

GPC (Chloroform + 0.1% TFA): M_n = 11 kDa, M_w = 15 kDa, PDI = 1.4. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.24 (**A**, d, J = 8.6 Hz, 24H), 7.15 (**B**, d, J = 8.5 Hz, 26H), 7.03 (**C**, d, J = 8.5 Hz, 24H), 6.99 (**D**, d, J = 8.5 Hz, 25H), 6.68 (**M**, s, 4H), 4.28 (**E**, t, J = 7.0 Hz, 28H), 3.58 (**F**, s, 25H), 3.54 (t, J = 7.2 Hz, 7H), 2.90 (**G**, t, J = 7.0 Hz, 28H), 2.55 (**H**, t, J = 7.5 Hz, 57H), 1.76 (**J**, p, J = 7.6, 6.4 Hz, 57H), 1.66 (**N**, p, J = 7.5 Hz, 7H), 1.56 (s, 5H), 1.45 (**K**, d, J = 15.6 Hz, 84H).

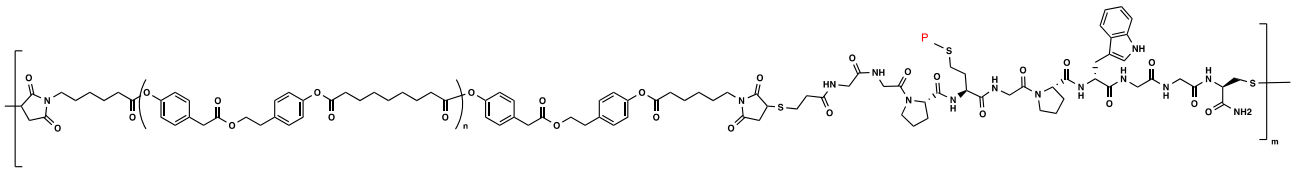


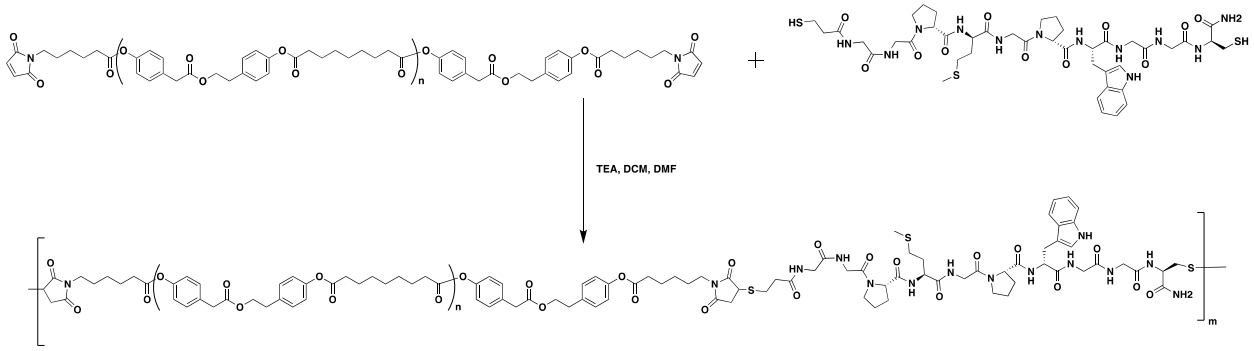


Scheme S2. Reaction schematic for synthesis of poly(HTy azelate) bismaleimide.

Synthesis of poly(HTy azelate-co-8%Pep)

GPC (Chloroform + 0.1% TFA): M_n = 142.1 kDa, M_w = 270.1 kDa, PDI = 1.9. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.24 (d, J = 8.5 Hz, 25H), 7.15 (d, J = 8.5 Hz, 26H), 7.02 (d, J = 8.5 Hz, 26H), 6.99 (d, J = 8.5 Hz, 26H), 6.68 (s, 1H), 4.28 (t, J = 7.0 Hz, 28H), 4.02 (hept, J = 6.1 Hz, 13H), 3.58 (s, 28H), 2.90 (t, J = 6.9 Hz, 27H), 2.55 (t, J = 7.5 Hz, 56H), 2.08 (P, s, 2H), 1.75 (q, J = 7.1 Hz, 58H), 1.65 (dt, J = 15.1, 7.4 Hz, 9H), 1.49 – 1.28 (m, 98H).





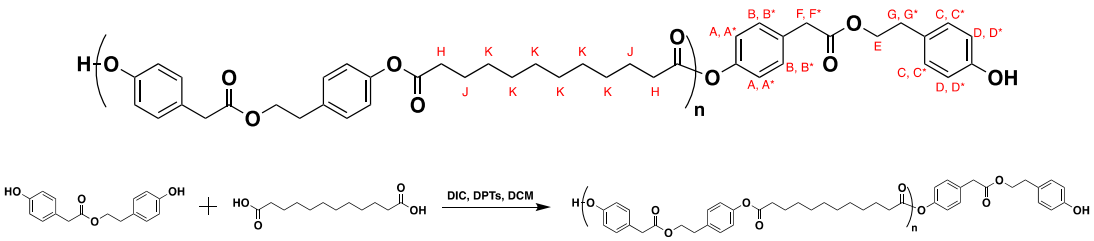
Scheme S3. Reaction schematic for synthesis of poly(HTy azelate-co-peptide).

Synthesis of poly(HTy dodecandioate) (r = 0.8)

GPC (Chloroform + 0.1% TFA): M_n = 7.0 kDa, M_w = 10.2 kDa, PDI = 1.5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.24 (A, d, J = 8.6 Hz, 20H), 7.20 (A*, d, J = 8.5 Hz, 4H), 7.15 (B, d, J = 8.5 Hz, 20H), 7.10 (B*, d, J = 8.5 Hz, 4H), 7.02 (C, d, J = 8.5 Hz, 20H), 6.99 (D, d, J = 8.5 Hz, 20H), 6.96 (C*, d, J = 8.5 Hz, 4H), 6.91 (D*, d, J = 8.4 Hz, 4H), 4.28 (E, t, J = 7.0 Hz, 20H), 3.58 (F, s, 20H), 3.54 (F*, s, 2H), 3.50 (F*, s, 2H), 2.90 (G, t, J = 7.0 Hz, 20H), 2.82 (G*, t, J = 6.6 Hz, 4H), 2.54 (H, t, J = 7.6 Hz, 63H), 1.75 (J, p, J = 7.5 Hz, 76H), 1.45-1.30 (K, m, 120H).

Synthesis of poly(HTy dodecandioate) (r = 0.95)

GPC (Chloroform + 0.1% TFA): M_n = 26.4 kDa, M_w = 45.6 kDa, PDI = 1.7. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.24 (A, d, J = 8.6 Hz, 98H), 7.19 (A*, d, J = 2.2 Hz, 5H), 7.15 (B, d, J = 8.5 Hz, 99H), 7.09 (B*, t, J = 2.1 Hz, 3H), 7.02 (C, d, J = 8.5 Hz, 100H), 6.98 (D, d, J = 8.5 Hz, 101H), 6.92 (C*, d, J = 8.6 Hz, 5H), 4.28 (E, t, J = 7.0 Hz, 116H), 3.58 (F, s, 105H), 3.54 (F*, s, 7H), 3.50 (F*, s, 3H), 2.90 (G, t, J = 7.0 Hz, 111H), 2.82 (G*, t, J = 6.8 Hz, 5H), 2.54 (H, t, J = 7.5 Hz, 224H), 1.75 (J, p, J = 7.5 Hz, 200H), 1.45 – 1.28 (K, m, 600H).



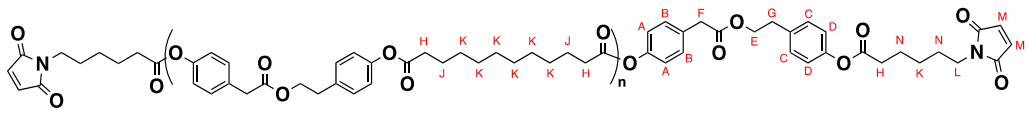
Scheme S4. Reaction schematic for synthesis of poly(HTy dodecanedioate).

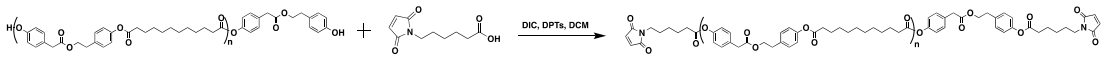
Synthesis of poly(HTy dodecanedioate) (r = 0.8) bismaleimide

GPC (Chloroform + 0.1% TFA): M_n = 8.8 kDa, M_w = 14 kDa, PDI = 1.6. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.24 (**A**, d, J = 8.5 Hz, 24H), 7.15 (**B**, d, J = 8.5 Hz, 24H), 7.02 (**C**, d, J = 8.5 Hz, 24H), 6.99 (**D**, d, J = 8.5 Hz, 22H), 6.68 (**M**, s, 4H), 4.28 (**E**, t, J = 7.0 Hz, 28H), 3.58 (**F**, s, 27H), 3.55 (t, J = 7.2 Hz, 7H), 2.90 (**G**, t, J = 6.9 Hz, 28H), 2.54 (**H**, t, J = 7.5 Hz, 56H), 1.78 – 1.72 (**J**, m, 48H), 1.66 (**N**, dt, J = 15.1, 7.5 Hz, 8H), 1.45 – 1.30 (**K**, m, 159H).

Synthesis of poly(HTy dodecanedioate) (r = 0.95) bismaleimide

GPC (Chloroform + 0.1% TFA): M_n = 33.0 kDa, M_w = 61.6 kDa, PDI = 1.89. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.24 (**A**, d, J = 8.5 Hz, 100H), 7.15 (**B**, d, J = 8.5 Hz, 99H), 7.03 (**C**, d, J = 8.5 Hz, 102H), 6.99 (**D**, d, J = 8.5 Hz, 103H), 6.68 (**M**, s, 4H), 4.29 (**E**, t, J = 7.0 Hz, 117H), 3.58 (**F**, s, 115H), 2.90 (**G**, t, J = 6.9 Hz, 116H), 2.55 (**H**, t, J = 7.5 Hz, 235H), 1.75 (**J**, p, J = 7.5 Hz, 238H), 1.46 – 1.29 (**K**, m, 728H)





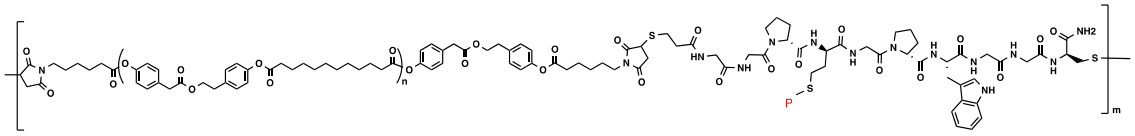
Scheme S5. Reaction schematic for synthesis of poly(HTy dodecanedioate) bismaleimide.

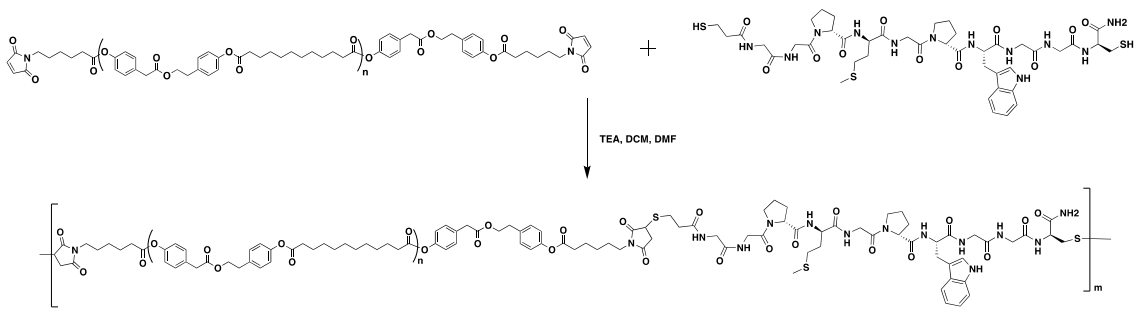
Synthesis of poly(HTy dodecanedioate-co-8%Pep)

GPC (Chloroform + 0.1% TFA): M_n = 63.0 kDa, M_w = 140.4 kDa, PDI = 2.2. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.24 (A, d, J = 8.8 Hz, 19H), 7.15 (B, d, J = 8.8 Hz, 24H), 7.02 (C, d, J = 8.7 Hz, 22H), 6.98 (D, d, J = 8.7 Hz, 25H), 6.68 (M, s, 1H), 4.28 (E, t, J = 7.3 Hz, 34H), 3.58 (F, s, 36H), 2.90 (G, t, J = 7.4 Hz, 28H), 2.54 (H, t, J = 8.0 Hz, 56H), 2.08 (P, s, 3H), 1.75 (J, dt, J = 15.7, 7.6 Hz, 45H), 1.48 – 1.18 (K, m, 156H).

Synthesis of poly(HTy dodecanedioate-co-2%Pep)

GPC (Chloroform + 0.1% TFA): M_n = 76.7 kDa, M_w = 112.8 kDa, PDI = 1.5. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.24 (A, d, J = 8.5 Hz, 97H), 7.15 (B, J = 8.5 Hz, 99H), 7.02 (C, d, J = 8.6 Hz, 97H), 6.99 (D, d, J = 8.5 Hz, 99H), 6.68 (M, s, 1H), 4.28 (E, t, J = 7.0 Hz, 107H), 3.58 (F, s, 107H), 2.90 (G, t, J = 7.0 Hz, 104H), 2.54 (H, t, J = 7.5 Hz, 212H), 2.09 (P, s, 3H), 1.75 (J, p, J = 7.5 Hz, 209H), 1.45 – 1.28 (K, m, 624H).

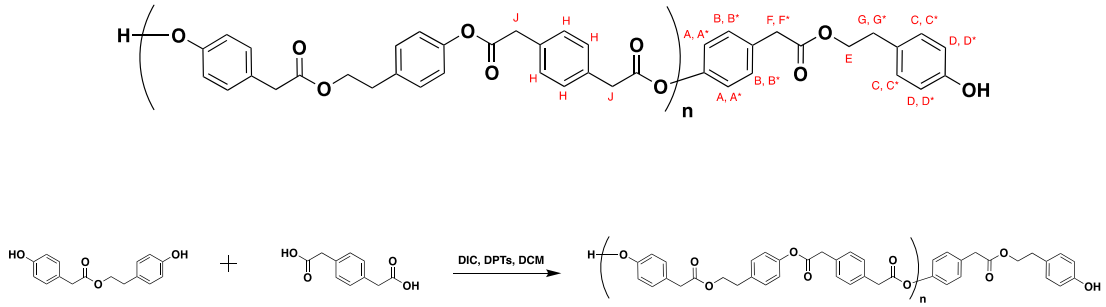




Scheme S6. Reaction schematic for synthesis of poly(HTy dodecanedioate-co-peptide).

Synthesis of poly(HTy phenylenediacetate) (r = 0.8)

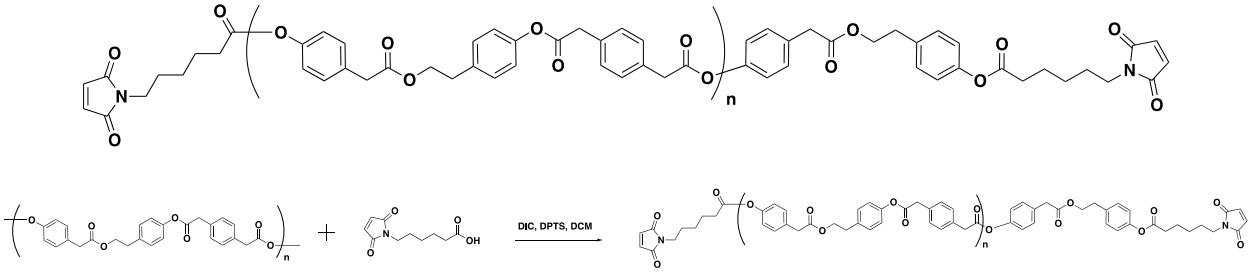
GPC (Chloroform + 0.1% TFA): M_n = 3.3 kDa, M_w = 5.9 kDa, PDI = 1.8. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.38 (H, d, J = 5.9 Hz, 61H), 7.21 (A, d, J = 8.4 Hz, 18H), 7.17 (A*, d, J = 8.7 Hz, 5H), 7.11 (B, d, J = 8.5 Hz, 18H), 7.06 (B*, d, J = 8.5 Hz, 4H), 7.01 (C, d, J = 8.5 Hz, 21H), 6.97 (D, d, J = 8.4 Hz, 23H), 6.92 (C*, d, J = 8.6 Hz, 3H), 6.90 (D*, d, J = 8.4 Hz, 5H), 4.26 (E, t, J = 6.9 Hz, 29H), 3.84 (J, s, 50H), 3.55 (F, s, 26H), 3.48 (F*, s, 3H), 2.87 (G, t, J = 6.8 Hz, 25H).



Scheme S7. Reaction schematic for synthesis of poly(HTy phenylenediacetate).

Synthesis of poly(HTy phenylenediacetate) (r = 0.8) bismaleimide

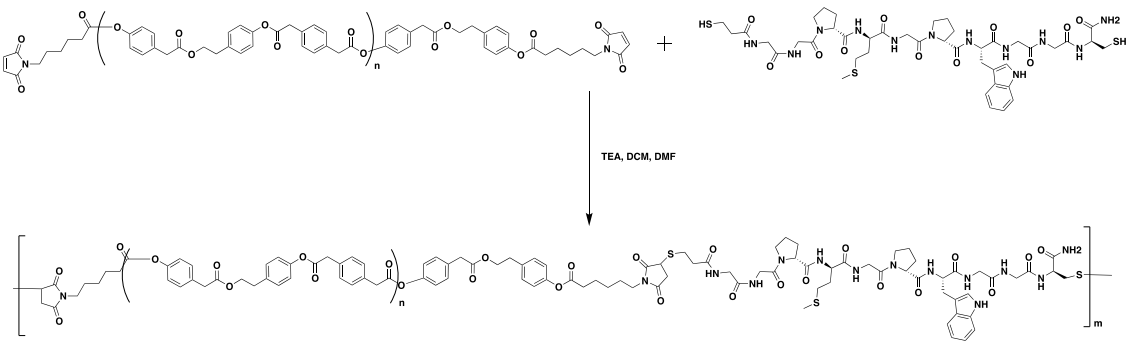
GPC (Chloroform + 0.1% TFA): M_n = 9.2 kDa, M_w = 12.8 kDa, PDI = 1.4. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.37 (s, 41H), 7.21 (d, J = 8.4 Hz, 20H), 7.12 (d, J = 8.4 Hz, 20H), 7.01 (d, J = 8.5 Hz, 21H), 6.97 (d, J = 8.4 Hz, 23H), 6.67 (s, 4H), 4.26 (t, J = 7.4 Hz, 26H), 3.84 (s, 44H), 3.56 (s, 33H), 2.88 (t, J = 6.8 Hz, 26H), 2.54 (t, J = 7.4 Hz, 7H), 1.77 (p, J = 7.5 Hz, 7H), 1.66 (p, J = 7.4 Hz, 7H), 1.42 (q, J = 8.1 Hz, 8H)



Scheme S8. Reaction schematic for synthesis of poly(HTy phenylenediacetate) bismaleimide.

Synthesis of poly(HTy phenylenediacetate-co-8%Pep)

GPC (Dimethylformamide + 0.1% TFA): M_n = 50.6 kDa, M_w = 96.7 kDa, PDI = 1.9. ^{1}H NMR (500 MHz, DMSO-d6) δ 7.36 (s, 17H), 7.22 (t, J = 8.3 Hz, 20H), 7.03 (dd, J = 14.0, 8.5 Hz, 21H), 4.23 (s, 10H), 3.94 (s, 17H), 3.63 (s, 10H), 2.86 (s, 11H), 2.01 (s, 2H), 1.67 – 1.56 (m, 3H), 1.51 (s, 3H), 1.31 (s, 3H).



Scheme S9. Reaction schematic for synthesis of poly(HTy phenylenediacetate-co-peptide).

NMR Spectra

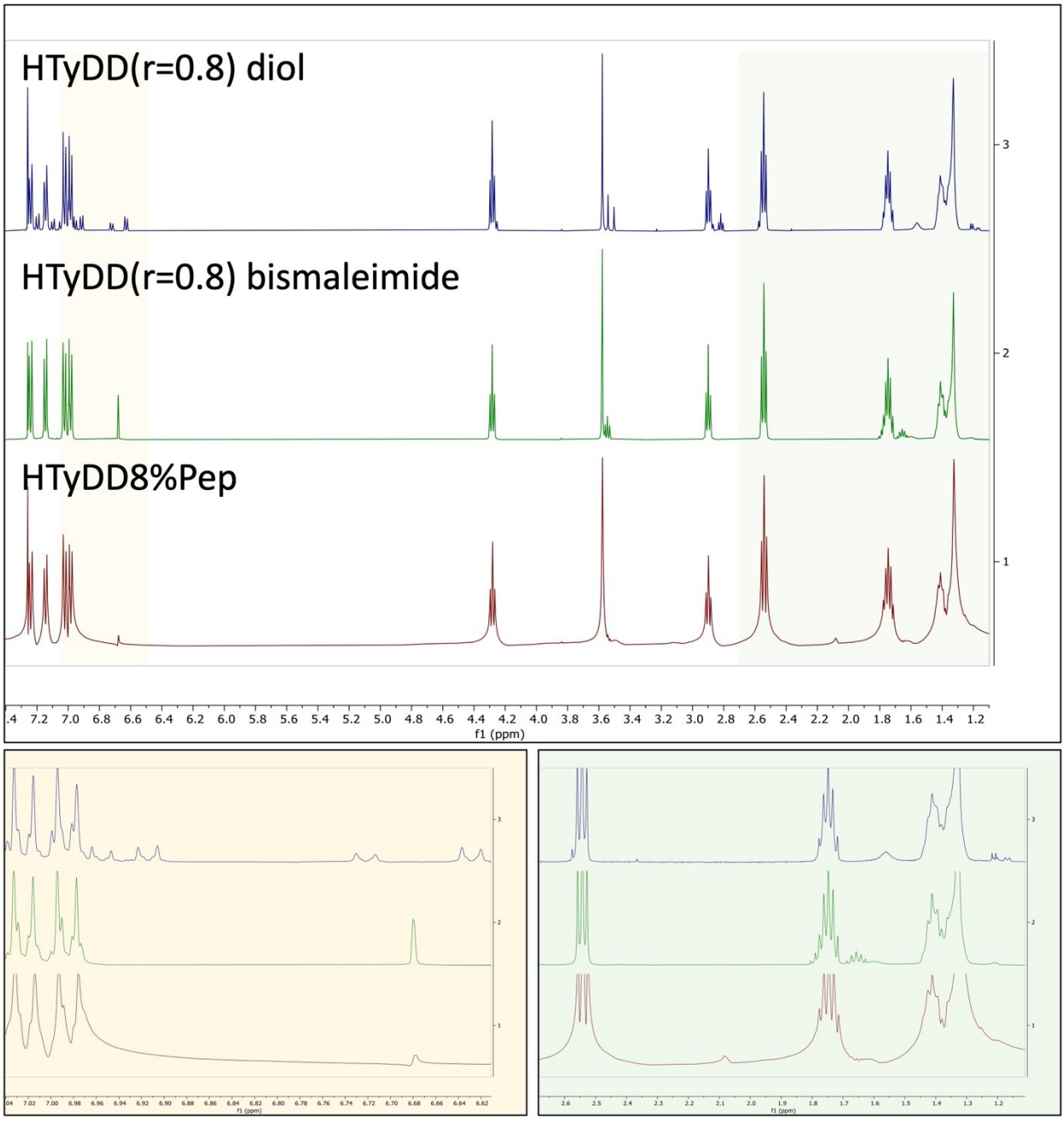


Figure S1. Structural confirmation of HTyDD (r = 0.8), HTyDD (r = 0.8) bismaleimide, and HTyDD8%Pep by ^{1}H NMR in $CDCl_3$. Maleimide peaks designated at 6.68 ppm (yellow) and methionine (peptide) peak designated at 2.09 ppm (green).

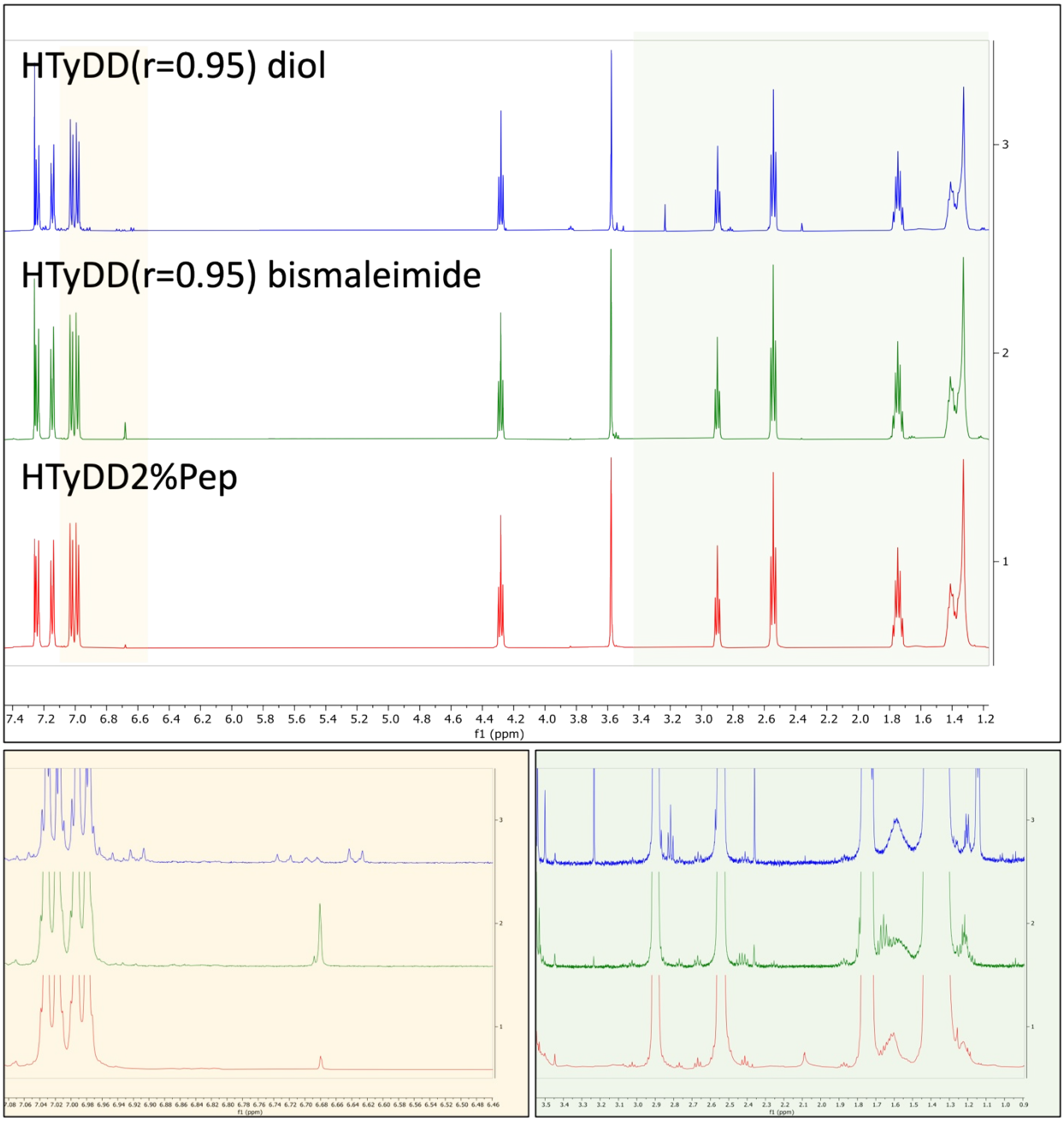


Figure S2. Spectral confirmation of HTyDD (r = 0.95), HTyDD (r = 0.95) bismaleimide, and HTyDD2%Pep by ^{1}H NMR in $CDCl_3$. Maleimide peaks designated at 6.68 ppm (yellow) and methionine (peptide) peak designated at 2.09 ppm (green).

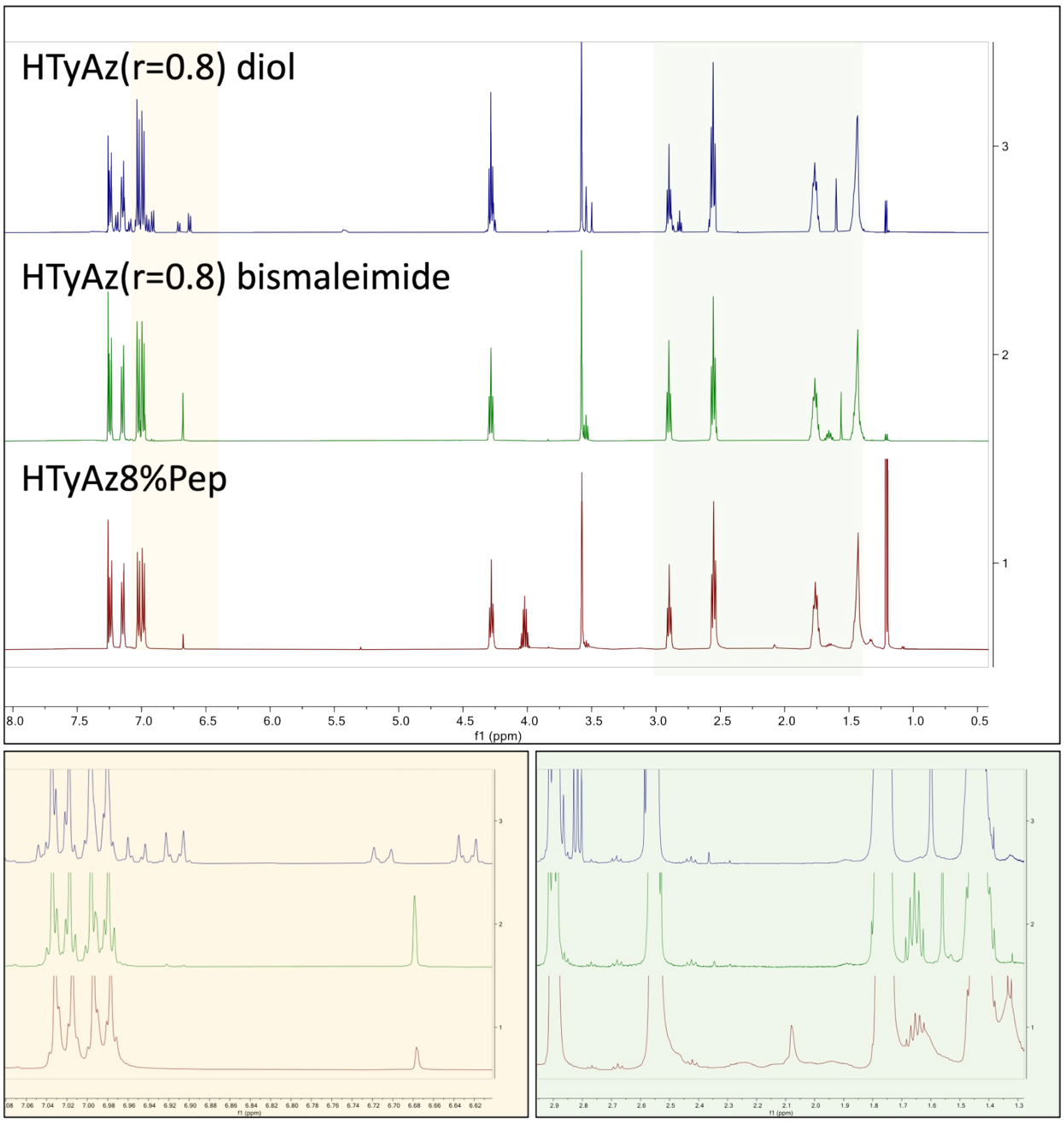


Figure S3. Spectral confirmation of HTyAz (r = 0.8), HTyAz (r = 0.8) bismaleimide, and HTyAz8%Pep by ^{1}H NMR in $CDCl_3$. Maleimide peaks designated at 6.68 ppm (yellow) and methionine (peptide) peak designated at 2.09 ppm (green).

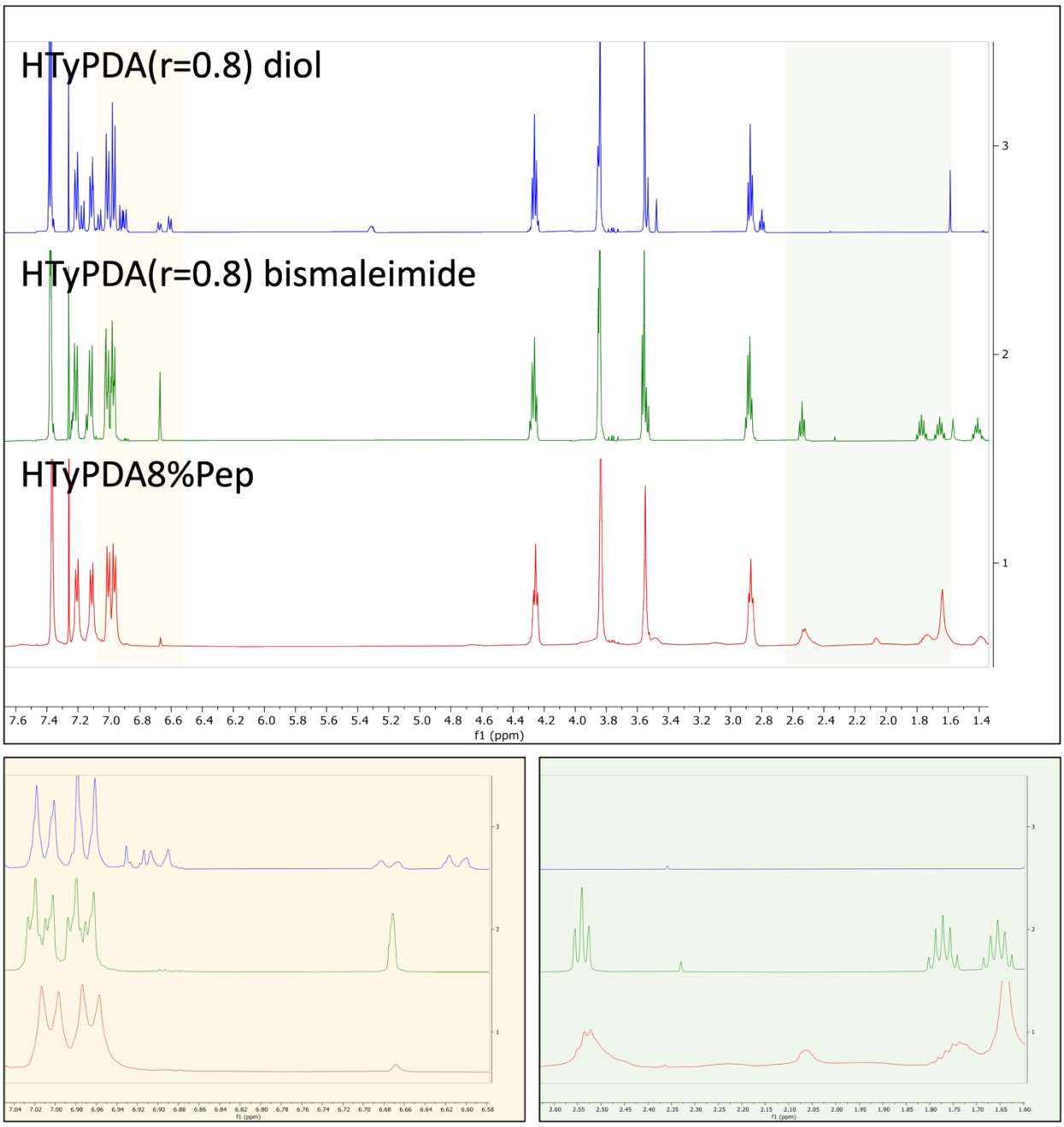


Figure S4. Spectral confirmation of HTyPDA (r = 0.8), HTyPDA (r = 0.8) bismaleimide, and HTyPDA8%Pep by ^{1}H NMR in $CDCl_3$. Maleimide peaks designated at 6.68 ppm (yellow) and methionine (peptide) peak designated at 2.09 ppm (green).

Peptide validation

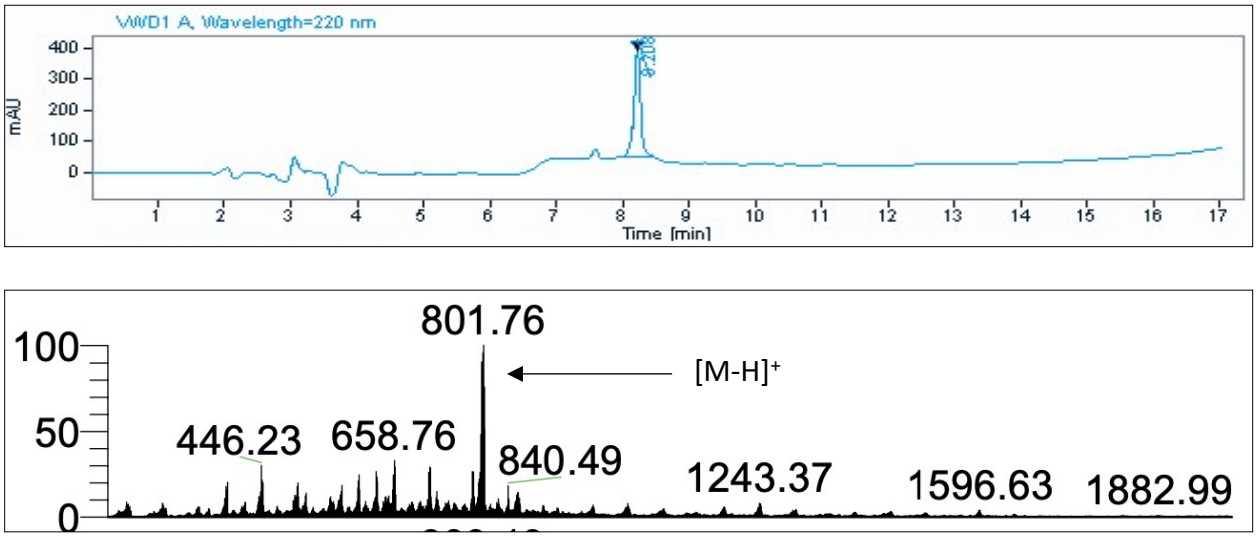


Figure S5. Purity (top) & mass conformation (bottom) of control peptide

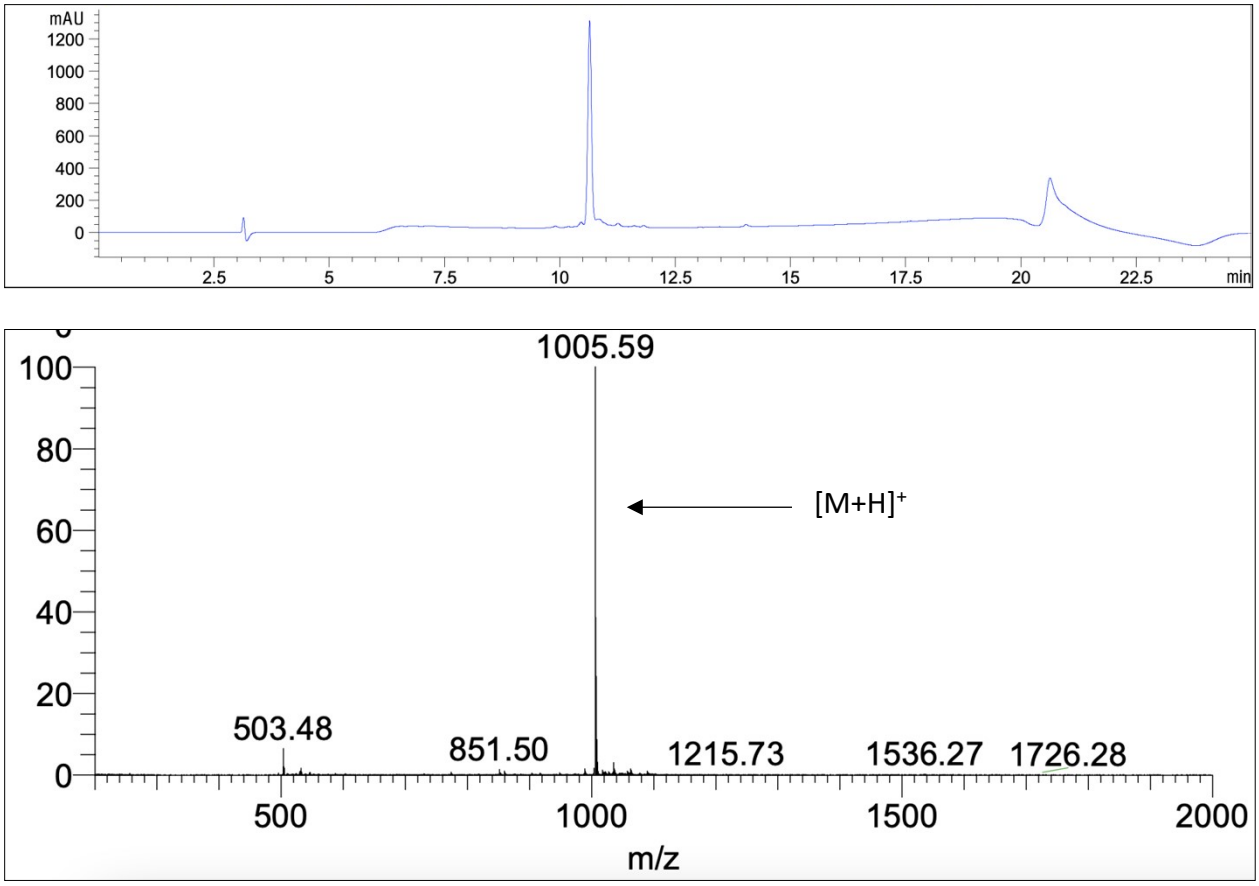


Figure S6. Purity (top) and mass confirmation (bottom) of degradable peptide

Differential Scanning Calorimetry

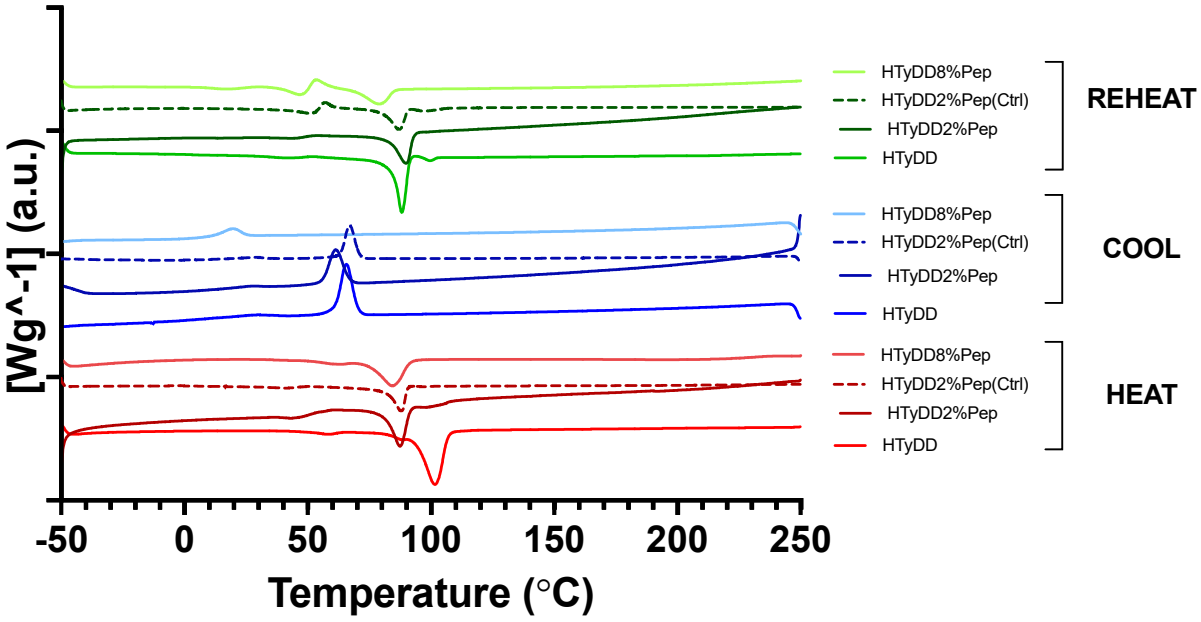


Figure S7. Differential scanning calorimetry thermograms of HTyDD, HTyDD2%Pep(Ctrl), HTyDD2%Pep, and HTyDD8%Pep

Thermogravimetric Analysis

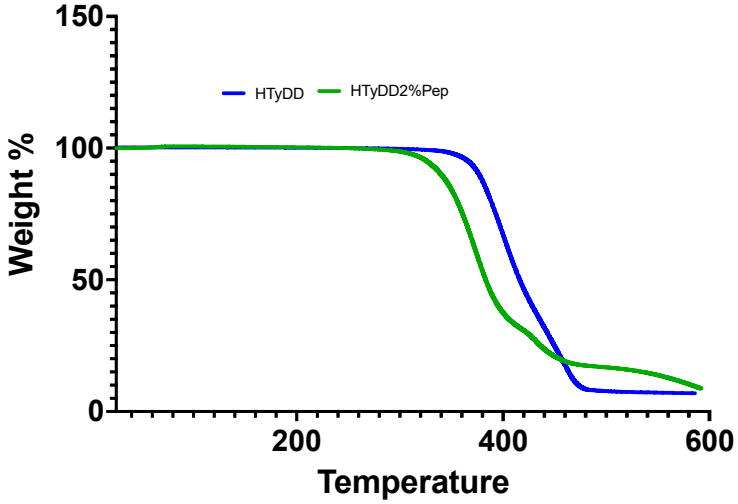


Figure S8. Thermogravimetric analysis of HTyDD and HTyDD2%Pep polymers

XRD Crystallinity Calculations

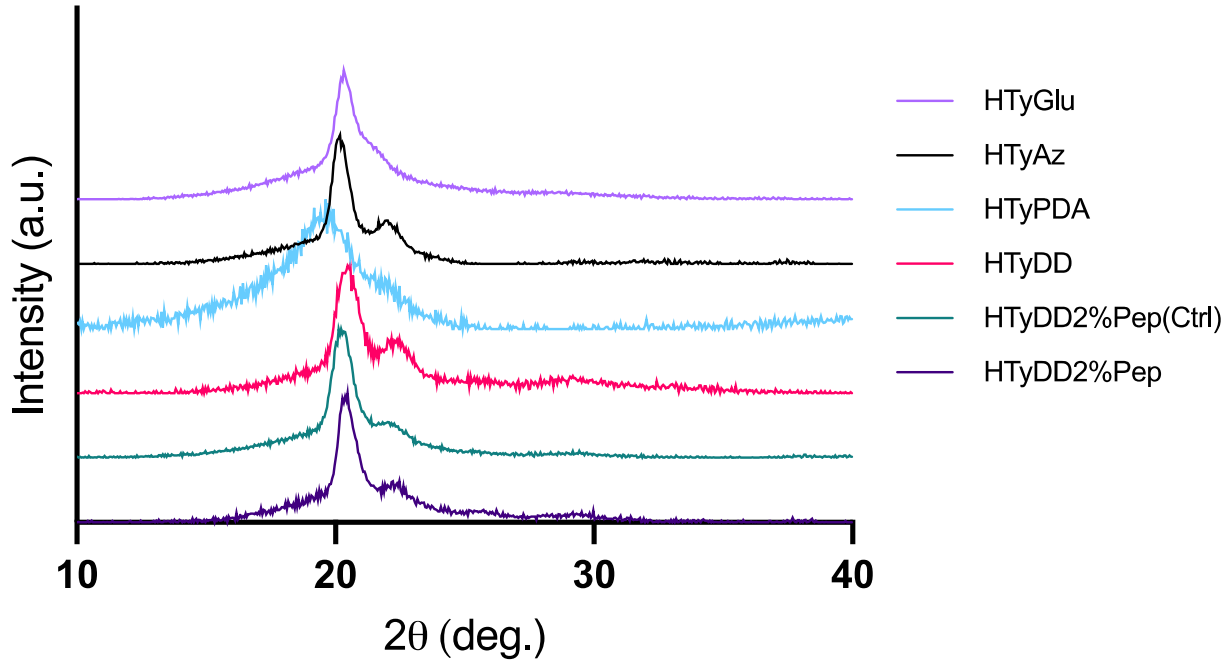


Figure S9. XRD spectral analysis

To calculate polymer crystallinity and crystallite size, XRD data was collected, and JADE software used to profile fit the scans. Representative profile fitted scans are shown in Figure S10. For each polymer spectra, a minimum number of peaks was used for profile fitting. For each fitted peak, the full-width half-max (FWHM), 2θ, and peak-area were recorded. Peaks with a width >3° at FWHM were considered amorphous while <3° at FWHM were considered crystalline. For each sample, peaks >28° were not considered to contribute to the polymer and were therefore not included in the calculations. Polymer crystallinity was calculated by adding the peak area associated with the crystalline peaks and dividing by the total peak area from all fitted peaks.

Polymer crystallite size (τ) was calculated using the Scherrer equation:

$$\tau = \frac{K\lambda}{\beta \cos\theta} \quad \text{(S1)}$$

where K is the dimensionless shape factor (0.9), λ is the X-ray wavelength (1.54), β is the FWHM (in radians), and θ is half of the measured two-theta peak angle (2θ in radians). All samples except for HTyPDA were composed of two main peaks at ~20 and ~23 deg. with the peak at ~20 deg. being the larger peak both in peak height and area and was therefore considered the most representative polymer peak. When calculating the crystallite size, only this larger peak was used in the calculations. The polymer crystallite size was calculated as the weighted average of the crystallite size associated with each fitted crystalline peaks where each peaks weighted contribution was determined from the area of that peak divided by the total area of all crystalline peaks. An example of this can be seen in Figure S1A, where the polymer peak at ~20 deg. is the most representative polymer peak. This peak has two crystalline profile fitted peaks at 20.20 and 20.63 deg. with crystallite sizes of 137 and 100 Å, representatively, and peak areas of 6086 and 12279, representatively. The weighted average crystallite size (τ_w) was calculated as:

$$\tau_w = 137 * \frac{6086}{6086 + 12279} + 100 * \frac{12279}{6086 + 12279} = 112 \text{ Å} \quad \text{(S2)}$$

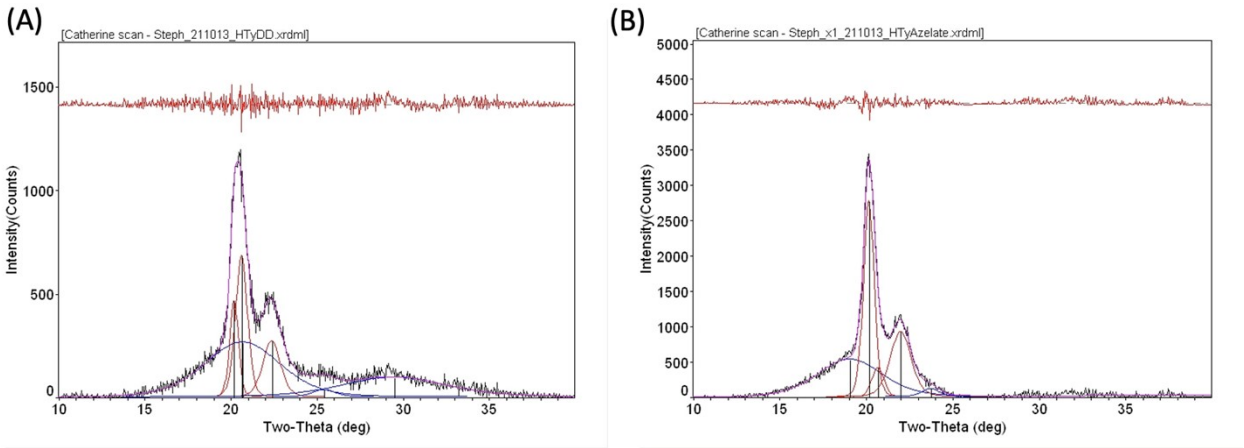
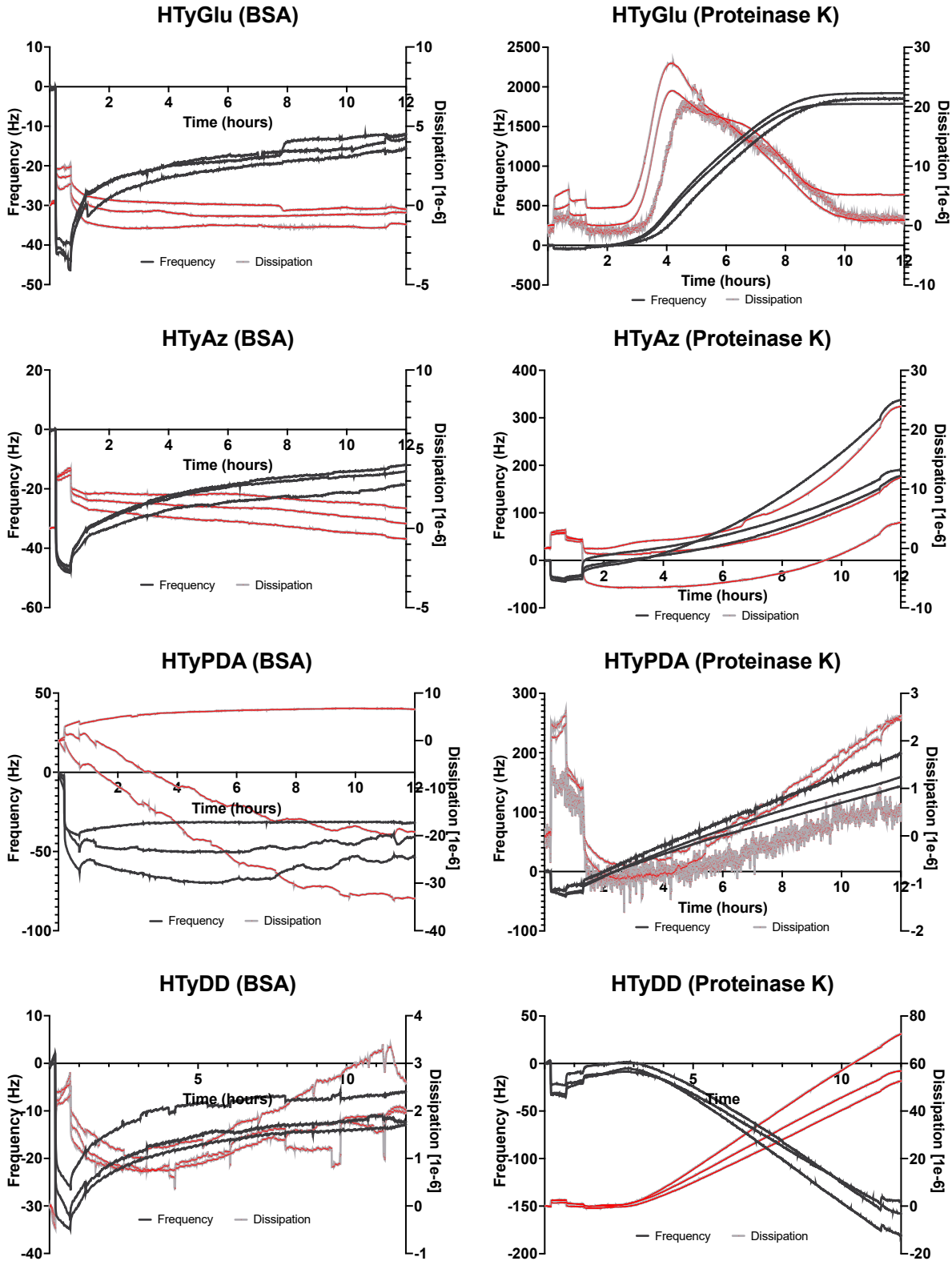


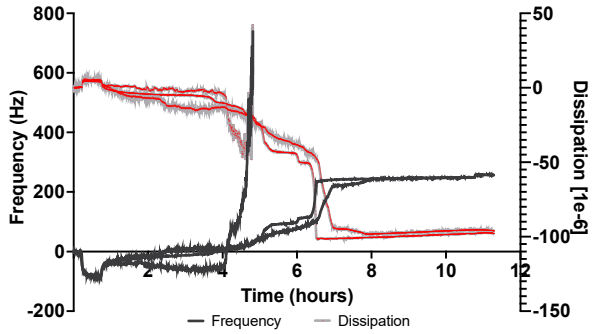
Figure S10. Representative XRD scans of (A) HTyDD and (B) HTyAzelate with applied profile fittings. The residual plot at the top of the spectra in red represents the calculated differences

between the applied fitting and the raw data. Crystalline peaks are shown in red and amorphous peaks in blue with the pink curve representing the sum of the individual components. For HTyDD (A), the peaks at 20.20 and 20.63 deg. represent the polymer crystalline contributions and were therefore used in a weighted average calculation for the polymer's crystallite size. For HTyAzelate (B), the peak at 20.17 deg. was the most representative crystalline peak and therefore was used in the calculation for the polymer's crystallite size.

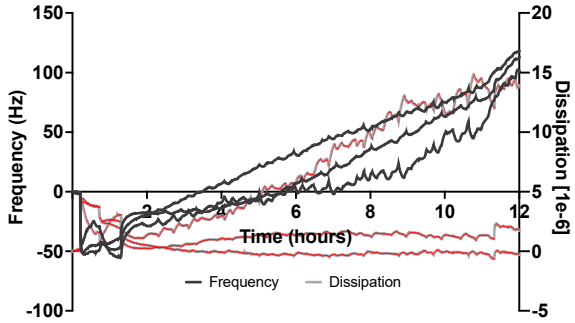
Quartz Crystal Microbalance with Dissipation (QCM-D) Frequency and Dissipation



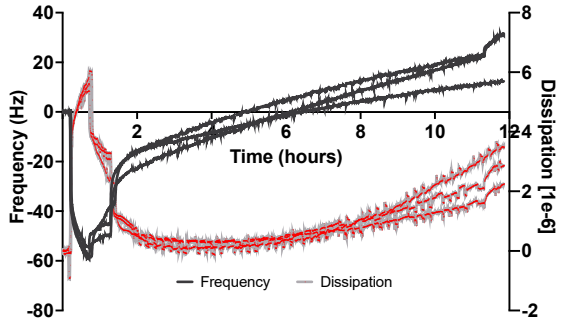
HTyAz8%Pep (Proteinase K)



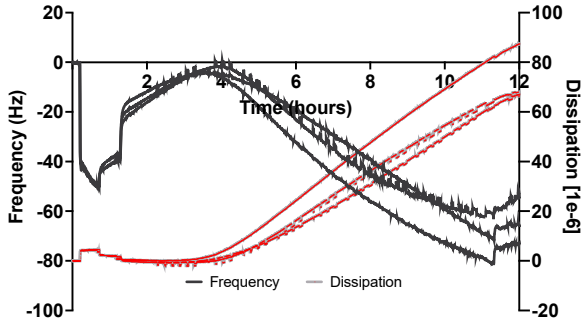
HTyPDA8%Pep (Proteinase K)



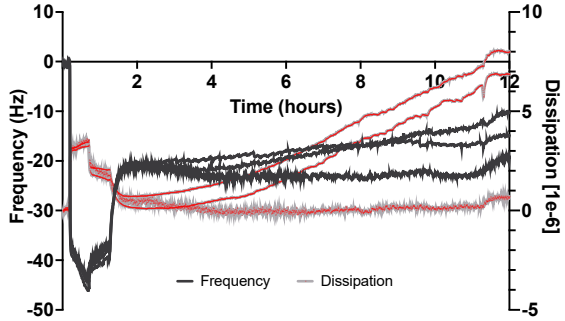
HTyDD8%Pep (Proteinase K)



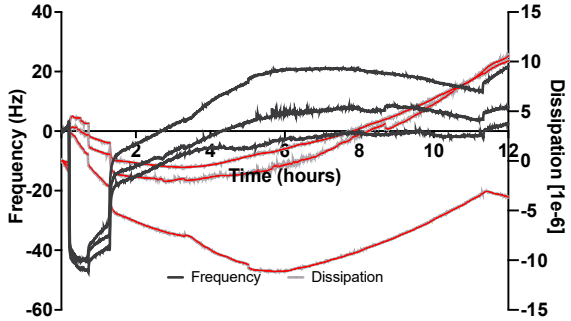
HTyDD2%Pep(Ctrl) (Proteinase K)



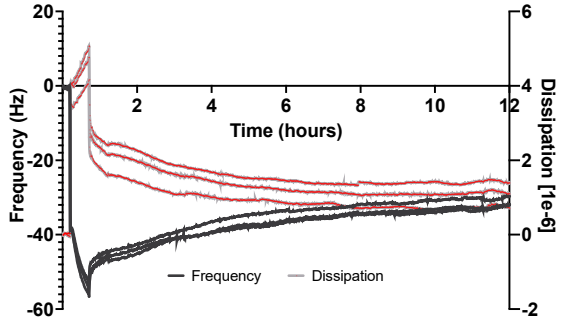
HTyDD2%Pep (BSA)



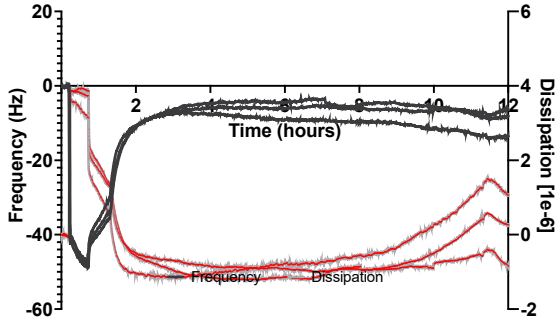
HTyDD2%Pep (Proteinase K)



HTyDD2%Pep (Lipase)



HTyDD2%Pep (Trypsin)



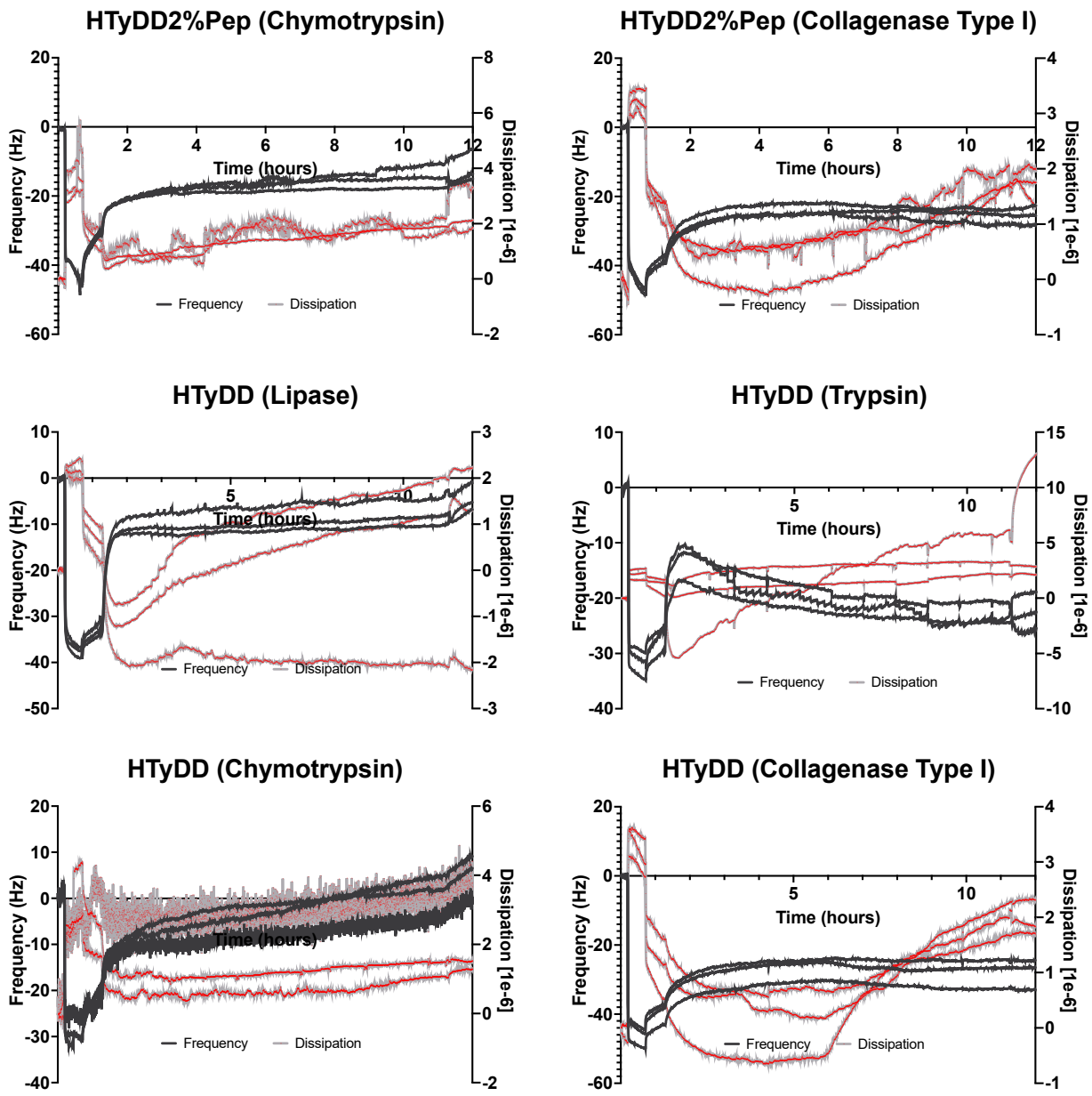


Figure S11. Frequency (dark grey) and dissipation (light grey) measured via QCM-D as a function of time. Data is represented as (n = 3) sensors per graph.