Controlled polymerization of histidine and synthesis of well-defined stimuli responsive polymers. Elucidation of the structure-aggregation relationship of this highly multifunctional material

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Electronic Supplementary Information

Experimental Section.

Materials

Boc-His(*Trt*)-OH (>99 %) was purchased from SENNCHEM. Triphosgene (99 %) was purchased from Acros Organics. Sarcosine (H-Sar-OH) (>99 %), H-Glu-OH (>99 %) and H-Leu-OH (>99 %) from Aldrich. Triethylamine (>99 %, Acros Organics) was dried over calcium hydride for one day and then distilled and stored in the vacuum line over sodium. The appropriate quantity needed was freshly distilled in a vacuum line. Thionyl chloride (99.9% Aldrich) was fractionally distilled under high vacuum and was stored in sealed ampoules. L-lysine (99.9% Aldrich) was used as received. Purification of THF (dried, max 0.005 % water, Merck) was performed using standard high vacuum techniques reported elsewhere[1]. Ethyl acetate (>99.5 %, Merck) was fractionally distilled over phosphorous pentoxide. Hexane (>99 %, Merck) was fractionally distilled over sodium. Anhydrous dimethylamine (DMA) (>99.9 %,

Aldrich, b.p. 7 ^{o}C) was condensed under high vacuum at -78 ^{o}C and was treated with sodium hydroxide pellets at room temperature for one day, and was subsequently distilled into precalibrated ampules with break-seals. It was then diluted with acetonitrile to the appropriate concentration in a sealed apparatus equipped with precalibrated ampules and kept away from light. End-functionalized monoamino poly(ethylene oxide) with molecular weights 9.95 x 10^3 g/mol were purchased from Aldrich and were used as monofunctional macroinitiators.

Methods

Size Exclusion Chromatography. Size-exclusion chromatography (SEC) was used to determine the M_n and M_w/M_n values. The analysis was performed using two SEC sets. The one was composed of a Waters Breeze instrument equipped with a 2410 differential refractometer and a Precision PD 2020 two angles (15^0, 90^0) light scattering detector. The carrier solvent was a 0.10 % TFA (v/v) M solution of water/acetonitrile (60/40 v/v) at a flow rate of 0.8 mL/min at 35 ^{o}C. Three linear Waters hydrogel columns were used. For protected polymers, the analysis was performed using a second SEC equipment. The system was composed of a Waters 600 high pressure liquid chromatographic pump, Waters Ultrastyragel columns (HR-2, HR-4, HR-5E and HR-6E), a Waters 410 differential refractometer detector and a Precision PD 2020 two angles (15^0, 90^0) light scattering detector at 60 ^{o}C. A 0.1N LiBr DMF solution was used as an eluent at a rate of 1 mL/min.

NMR Spectroscopy. ^{1}H NMR spectroscopy (300 MHz) was performed using a Varian Unity Plus 300/54 spectrometer. The spectra of the polymers were performed either in D_2O with DCl, deuterated DMSO or in CF_3COOD, while the spectra of the NCAs were taken in $CDCl_3$ at room temperature.

FTIR. FTIR measurements were performed with a Perkin Elmer Spectrum One instrument, in KBr pellets at room temperature, in the range 450-4000 cm^{-1}.

Circular Dichroism. Circular Dichroism was performed with a Jasco J-815 model, featuring a peltier model PTC-423S/15 thermo stabilizing system. The cell used was 1 mm Quartz Suprasil cell. Typical concentrations were about 3 x 10^{-4} g/ml.

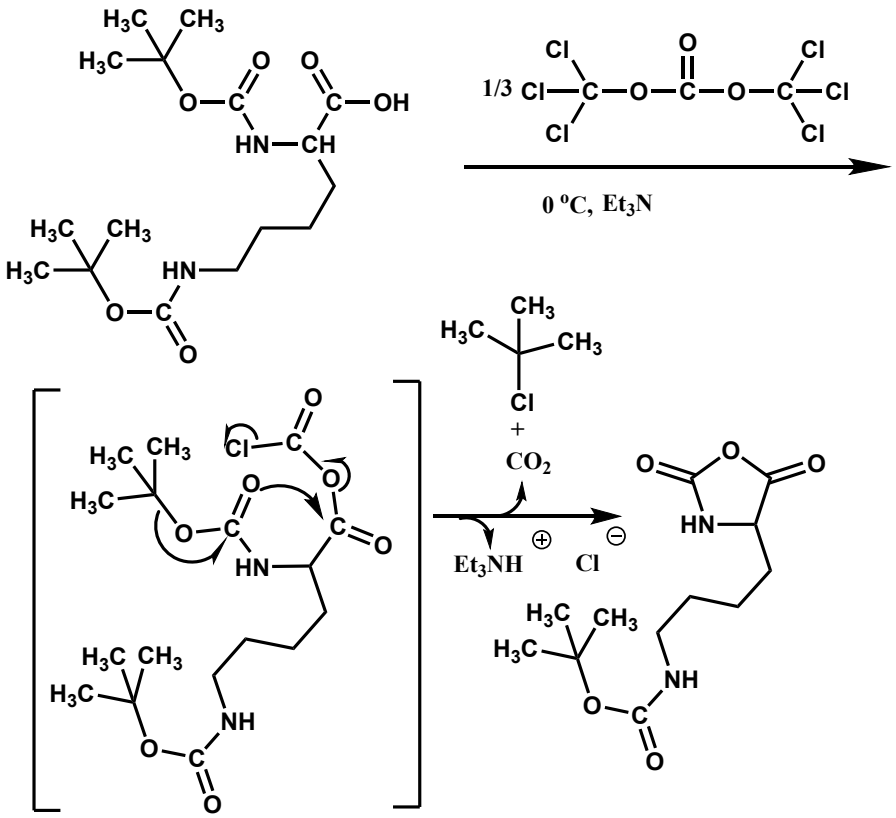
UV Spectroscopy. UV spectroscopy was performed with the Perkin Elmer Lamda 650 spectrometer, from 190-500 nm, at room temperature with cells requiring 120 µl.

Size measurements. Dynamic light scattering measurements were conducted with a Series 4700 Malvern system composed of a PCS5101 goniometer with a PCS7 stepper motor controller, a Cyonics variable power Ar+ laser, operating at 690 nm and with 10 mW power, a PCS8 temperature control unit, and a RR98 pump/filtering unit. Correlation functions were analyzed by the cumulant method and the Contin software. The correlation function was collected at 90°. All measurements were performed in isotonic Tris buffer (10 mM) at pH=7.4. The concentration range measured was between 2 x 10^{-3}- 1 x 10^{-5} g/ml.

Synthesis of the monomers

Synthesis of ε-*tert*-butyloxycarbonyl-L-lysine N-carboxy anhydride (BOCLL-NCA). *ε*-*tert*-Butyloxycarbonyl-L-lysine-NCA was synthesized according to previously reported method.[2] Briefly, N^{α}, N^{ε}-Di-(*tert*-butoxycarbonyl)-L-lysine was added into a flask, placed on the vacuum line and pumped overnight. Then purified ethyl acetate was distilled, followed by argon insertion in order to reach atmospheric pressure and by addition of triphosgene. The mixture was left to react for 10 minutes. Triethylamine diluted in dry ethyl acetate was subsequently added dropwise, and the solution was immersed in an ice-water bath for 6 hours. The precipitate was filtered, in order to remove the HCl salt of triethylamine, the clear solution was immersed in an ice bath, and the NCA was extracted with Milli-Q water repeatedly, until neutral pH of the aqueous phase was achieved. The purified NCA was recrystallized three times under high vacuum in a custom-made apparatus, with ethyl acetate/hexane (1/5 v/v) pair at –20 ^{o}C. The purity was confirmed by ^{1}H NMR along with FTIR spectroscopy.

The purity of BOCLL-NCA was confirmed by ^{1}H NMR and IR spectroscopy: ^{l}H NMR (300 MHz, $CHCl_3$, δ in ppm): 1.00-1.60 ppm (13H, 9 from BOC group -C($C\underline{H}_3$)$_3$, 4 from –HN-CH_2-$C\underline{H}_2$-$C\underline{H}_2$-CH_2-), 1.60-2.20 ppm (2H, –HN-CH_2-CH_2-CH_2-$C\underline{H}_2$-), 3.00-3.30, (2H, –HN-$C\underline{H}_2$-CH_2-CH_2-CH_2-), 4.20-4.40 (1H, N-C$\underline{H}$-CO), 4.40-5.00 ppm (1H, -CH-N$\underline{H}$-CO- of the anhydride ring), 6.80-7.20 ppm (1H, -CH_2-N$\underline{H}$-CO- of the ε-amine), IR (thin film): 1848, 1780 cm^{-1} (ν CO, NCA, s), 2650 cm^{-1} (ν CH from BOC group), 1690 cm^{-1} (ν CO, BOC protecting group). The reactions used for the synthesis of BOCLL-NCA are shown in Scheme S1, while its ^{1}H NMR spectrum is given in Figure F1. Typical yield was 65 %.



Scheme S1. Reactions used for the synthesis of BOCLL-NCA.

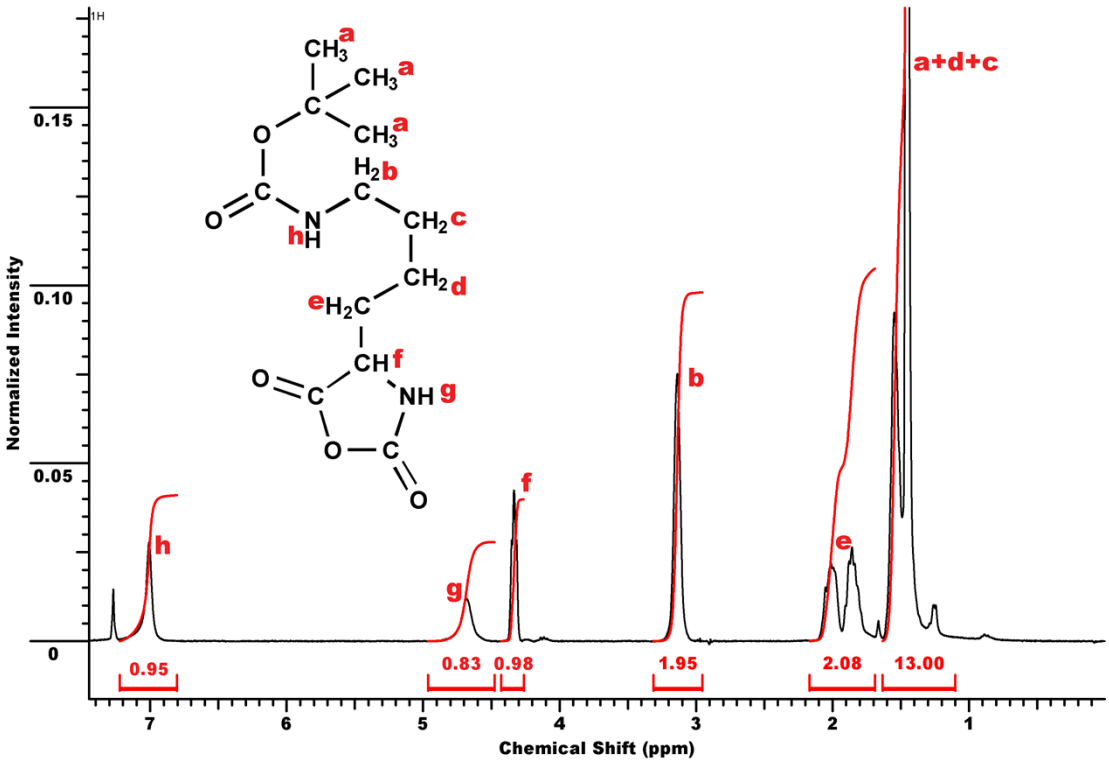
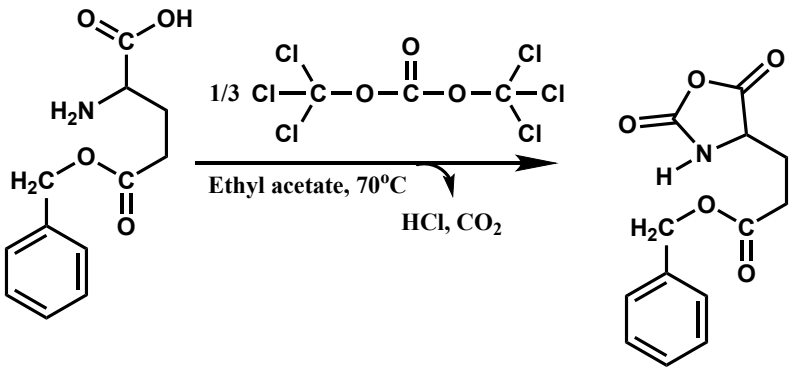


Figure F1. ^{1}H NMR spectrum of BOCLL-NCA.

Synthesis of γ-benzyl-L-glutamate N-carboxy anhydride (BLG-NCA)

The NCA of γ-*benzyl*-L-glutamate was synthesized according to the previously reported method.[3] Briefly, *γ-benzyl*-L-glutamate was suspended in dry ethyl acetate followed by addition of triphosgene. The mixture was heated at 70 ^{o}C until the solution became clear, indicating the formation of the NCA. The solvent was distilled off in the vacuum line, and fresh dry ethyl acetate was distilled in the flask, to dissolve the crude NCA, followed by removal of the solvent by distillation. This procedure was repeated twice in order to remove the excess phosgene that sublimes under high vacuum. The unreacted species, such as free amino acids along with the HCl salts of the amino acids produced during the synthesis, were removed by extraction with an alkali solution in water. The resulted BLG-NCA was further purified by three recrystallization from dry ethyl acetate/hexane (1/5 v/v) under high vacuum at -20 ^{o}C.

The yield of the formation of BLG-NCA was 65 %. The purity of BLG-NCA was confirmed by ^{1}H NMR and FTIR spectroscopy: ^{l}H NMR (300 MHz, $CHCl_3$, δ in ppm): 2.00-2.50 ppm (2H, -CH-C$\underline{H}_2$-CH_2-CO-), 2.50-2.70 ppm (2H, -CH-C$\underline{H}_2$-CH_2-CO-), 4.30-4.50, (1H, N-C$\underline{H}$-CO), 5.00-5.30 ppm (2H, Phenyl-C$\underline{H}_2$-O-CO-), 6.40-6.70 ppm (1H, -CH_2-N$\underline{H}$-CO- of the anhydride), 7.20-7.60 ppm (5H of phenyl group), IR (thin film): 1848, 1780 cm^{-1} (ν CO, NCA, s), 700 cm^{-1}, 748 cm^{-1} (ν =C-H out of plane bend, s), 1738 cm^{-1} (ν CO, ester, s). Details for characterization have been reported in our previous work.[3] The reactions used for the synthesis of the BLG-NCA are given at Scheme S2, while its ^{1}H NMR spectrum is given in Figure F2.



Scheme S2. Reactions used for the synthesis of the BLG-NCA.

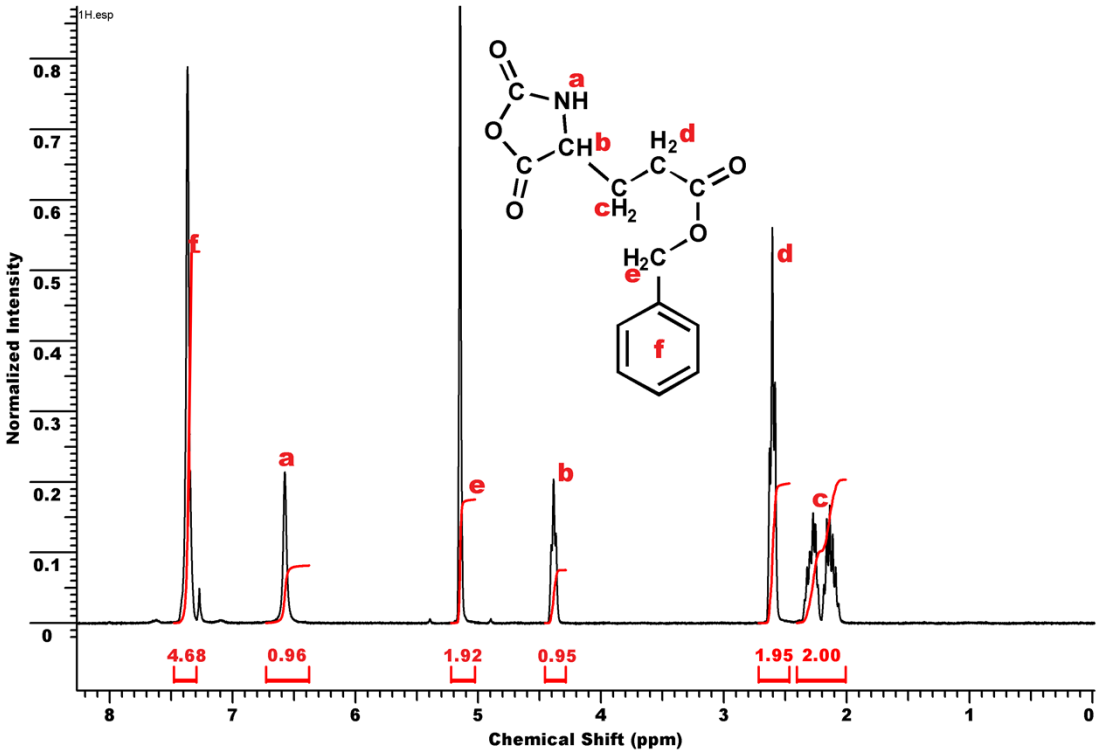


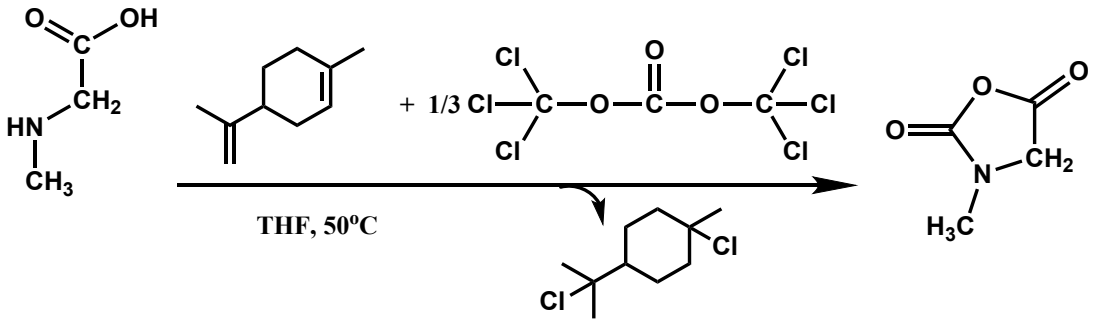
Figure F2. ^{1}H NMR spectrum of BLG-NCA.

Synthesis of sarcosine N-carboxy anhydride (SAR-NCA)

Synthesis was performed as previously reported with slight modifications.[4] 10 g (112 mmol) of finely grounded (critical for the course of reaction) sarcosine was dried

overnight in high vacuum in 500 ml round bottom flask. Subsequently, 250 ml THF distilled and 25 mL (145.6 mmol) of distilled (+)(-)limonene[5] were added to the flask forming a suspension. The reaction flask was then heated at 50 ^{o}C and 11 g (37.3 mmol) of equimolar amount of triphosgene were added, and the reaction was further heated up to 70 ^{o}C. After 1 hour, the solution became clear. After dissolution, the reaction was left another 30 minutes with a slight Argon flow and finally transferred to vacuum line and the solvent was distilled off giving a brown solid (Figure F3a). Once it was dried, SAR-NCA was sublimated at 60 ^{o}C overnight using a special custom made cold-finger glass apparatus under HV resulting in the formation of SAR-NCA crystals (Figure F3b). NCA was collected and sublimation repeated once again, until the peak at 1620 cm^{-1} corresponding to the HCl diadduct of limonene was completely eliminated (Figure F3c) yielding 8.8 g=76.5 mmol (68 %).

The yield was 68%. The purity of SAR-NCA was confirmed by ^{1}H NMR and IR spectroscopy (^{l}H NMR (300 MHz, $CHCl_3$, δ in ppm): 3.00-3.20, (3H, N-CH_3), 4.10-4.30 (2H, N-CH_2-CO), IR (thin film): 1848, 1780 cm^{-1} (νCO, NCA, s). The reactions used for the synthesis of SAR-NCA are shown in Scheme S3, while its FTIR and ^{1}H NMR spectra are provided in Figures F3 and F4, respectively.



Scheme S3. Reactions used for the synthesis of SAR-NCA.

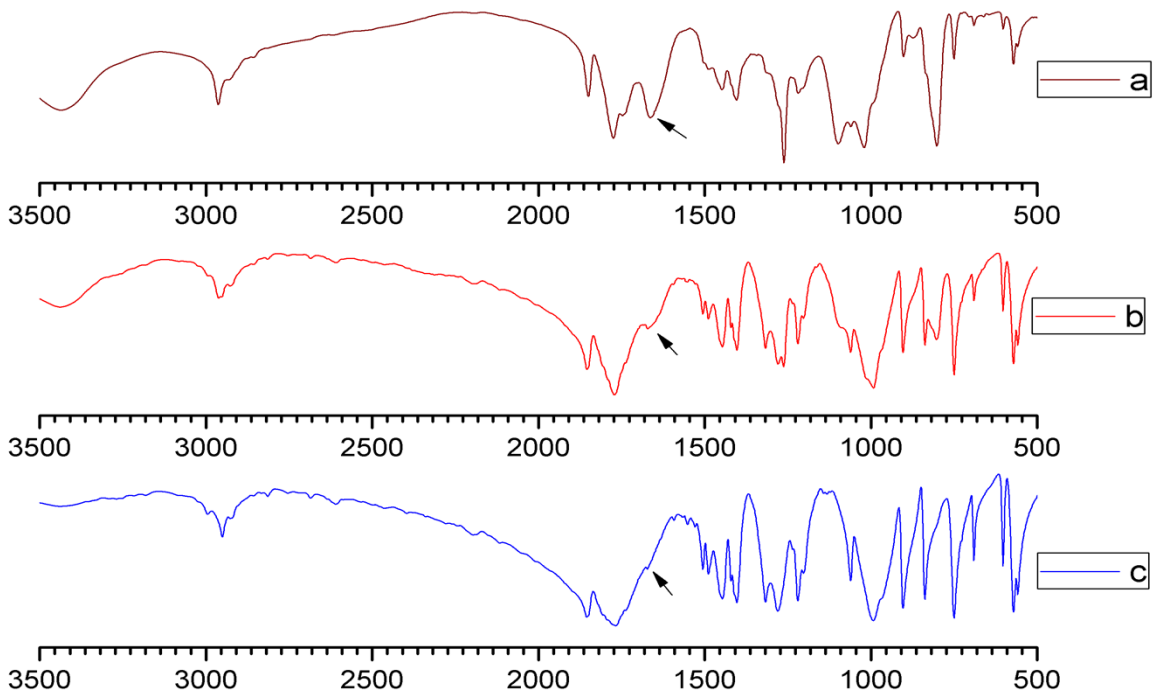


Figure F3: Monitoring the synthesis of SAR-NCA by FT-IR spectroscopy: a) Raw product after reaction, b) SAR-NCA after first and c) Second recrystallizations. In all spectra the peak at 1620cm^{-1} which is due to HCl substituted (+)-limonene is indicated.

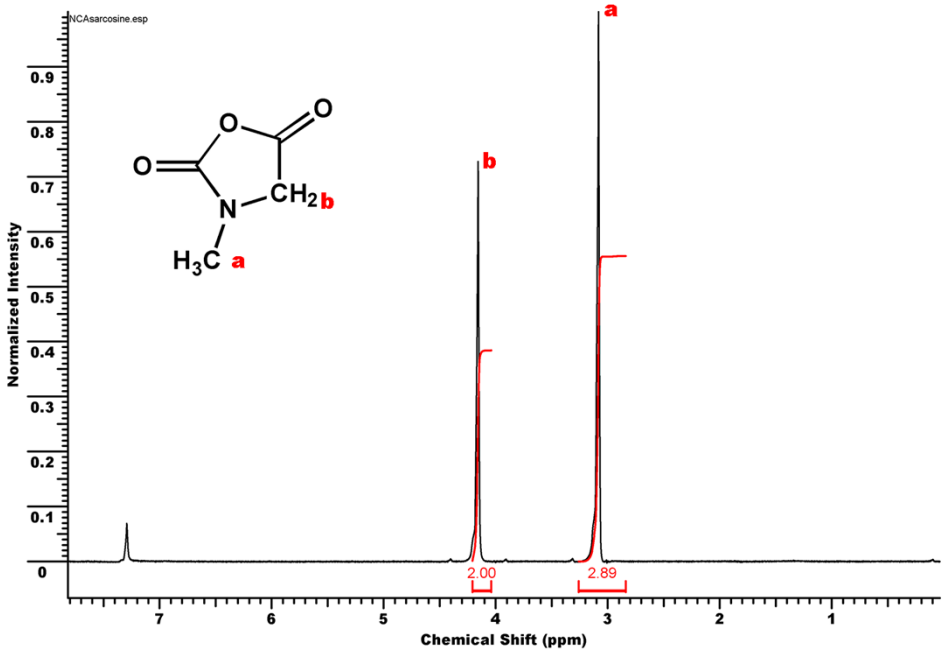
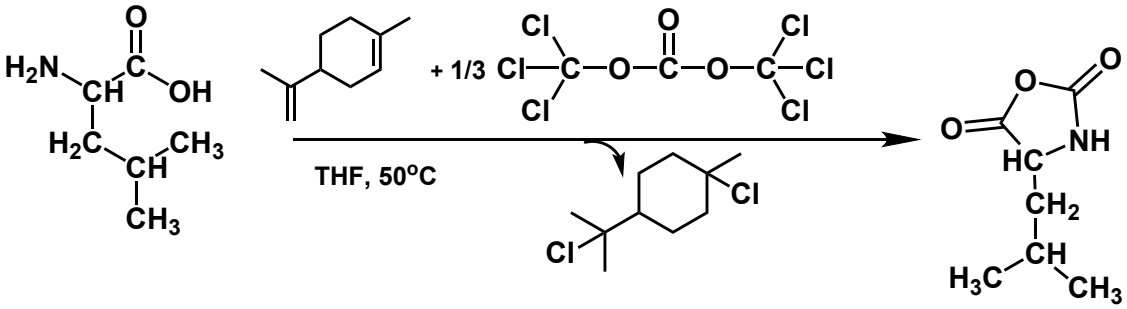


Figure F4. ^{1}H NMR spectrum of SAR-NCA.

Synthesis of leucine - N-carboxy anhydride (LEU-NCA)

The synthetic approach has been presented in our previous work.[6] In a flame dried 1 L two-neck round-bottom flask, 15 g, (114 mmol) of L-leucine were added and the apparatus was degassed under high vacuum (HV) overnight. Then, 250 mL of highly dry THF were distilled, the flask was removed from the vacuum line and was brought to atmospheric pressure by the careful addition of dry argon. The flask was equipped with a condenser and an inlet for Ar streaming. Under stirring, 29.6 mL, (183 mmol, 1.6 eq.) of purified (+)(-) limonene were was added and the suspension allowed to warm under vigorous stirring at 50-55 °C. At this time, 13.6 g (46 mmol, 1.2/3 eq.) of trisphosgene were added. The reaction mixture was allowed to stir at this temperature until a clear dark orange solution was formed (after 1-2 h). Then, the clear solution was stirred for another 1 h under Ar flow and the solution was transferred by filtration to the crystallization apparatus. The apparatus was attached to the vacuum line, and the solvent was pumped out to dryness. A small amount of highly dry THF (~20 mL) was then distilled in order to dissolve the solid monomer, until a clear solution was formed. Under vigorous stirring, n-hexane (~500 mL) was slowly distilled in order the NCA to precipitate in the form of a fine powder. The apparatus was kept at -20 °C overnight. The next day, the orange supernatant was filtered off and the solid was dried *in vacuo* for 1 h. Three additional crystallizations were performed and finally the white solid product was dissolved in dry THF and cannula transferred in a sealed flask. The flask was attached to the vacuum line, the

solvent removed and final product dried overnight. Finally, the flask was moved to a glove box and the solid was weighed yielding 16.6 g (95 mmol) of Leu-NCA (83 %).



Scheme S4. Reaction scheme for LEU-NCA.

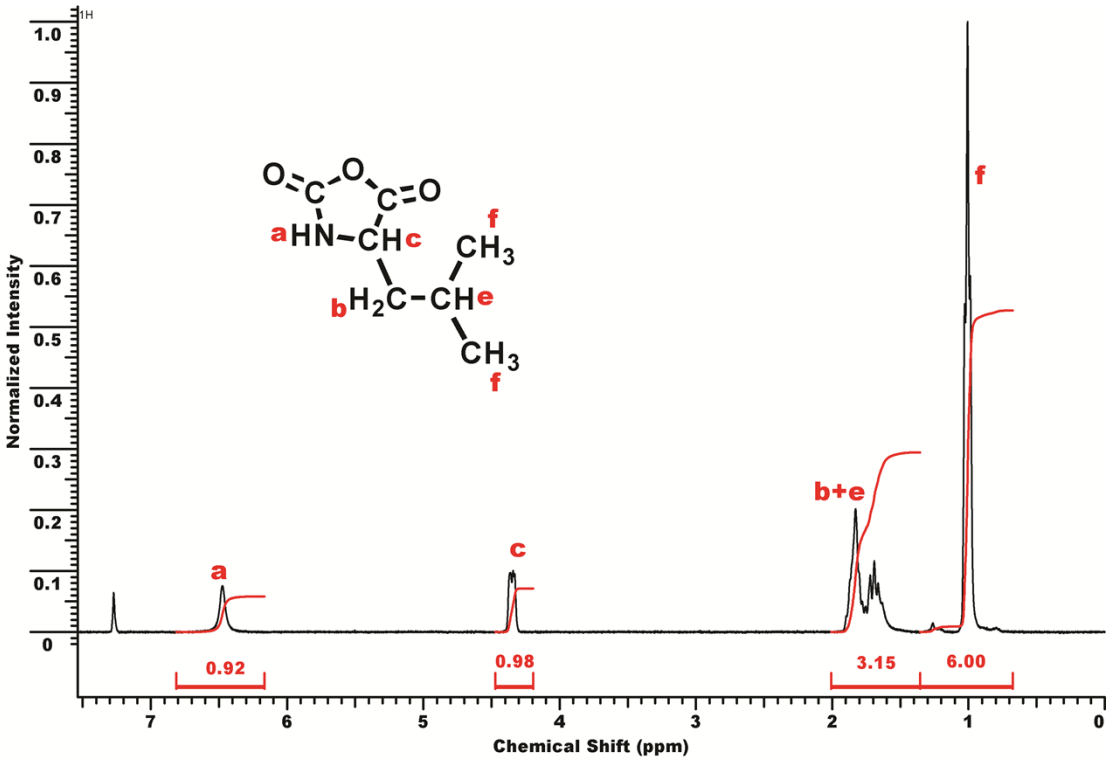


Figure F5. ^{1}H NMR spectrum of LEU-NCA.

The yield was 83%. The purity of the LEU-NCA was confirmed by ^{1}H NMR and IR spectroscopy (^{1}H NMR (300 MHz, $CHCl_3$, δ in ppm): 0.50-1.00, (6H, CH_2-CH-($C\underline{H}_3$)$_2$),

1.50-2.00 (3H, C$\underline{H}_2$-C$\underline{H}$-(CH_3)$_2$), 6.10-6.40 (1H, N$\underline{H}$ of NCA ring), IR (thin film): 3450 cm^{-1} (vNH), 1848, 1780 cm^{-1} (vCO, NCA, s).

The reactions used for the synthesis of LEU-NCA are illustrated in Scheme S4, while its ^1H NMR spectrum is given in Figure F5.

Kinetic studies of HIS-NCA polymerization

HIS-NCA polymerization kinetics with DMA as the initiator and DMF at room temperature

For the experiments concerning the kinetic studies, monomer conversion was monitored with FT-IR spectroscopy. Calibration samples were made in DMF over a concentration range f from 2.1 x 10^{-4} mol/ml to 3.1 x 10^{-6} mol/ml. Absorption spectra were taken focusing on the characteristic carbonyl peaks of NCA. The selected carbonyl peak of the NCA at 1850 cm^{-1} (Figure F7) gave better linearity compared to the peak at 1780 cm^{-1}. For all the calibration and polymerization samples, absorption spectra were taken after 10 scans, with 1 cm^{-1} resolution, normalized at 1900 cm^{-1} and the peak areas were calculated (Figure F6).

Periodically sampling allowed the determination of NCA concentration in time during various polymerizations.

For the polymerization experiments a solution of DMA in freshly distilled DMF was used as initiator and kept under high vacuum. Custom-made reaction flasks of 250 ml were used, previously flame dried under HV, transferred to a glove-box and predetermined amounts of monomer and solvent were added. The monomer

concentration was kept constant for all polymerizations while the volume of initiator and DMF was changed for the different degrees of polymerization (20, 50, 100, 200). Although we applied cycles of HV-argon during sampling, the final volumes were not found to be significantly different from the initial volumes.

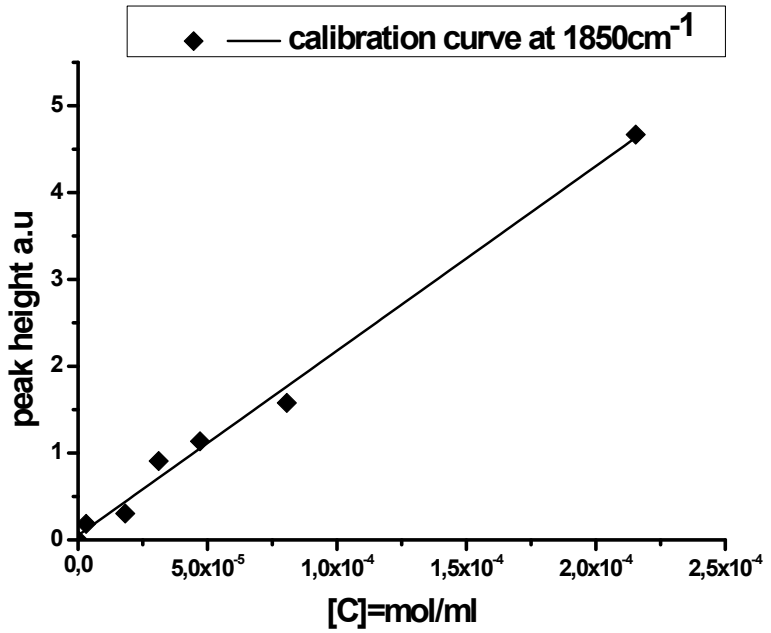


Figure F6. Calibration curve of the concentration of HIS-NCA using the peak at 1850 cm^{-1} as a function of peak height, obtained by FT-IR.

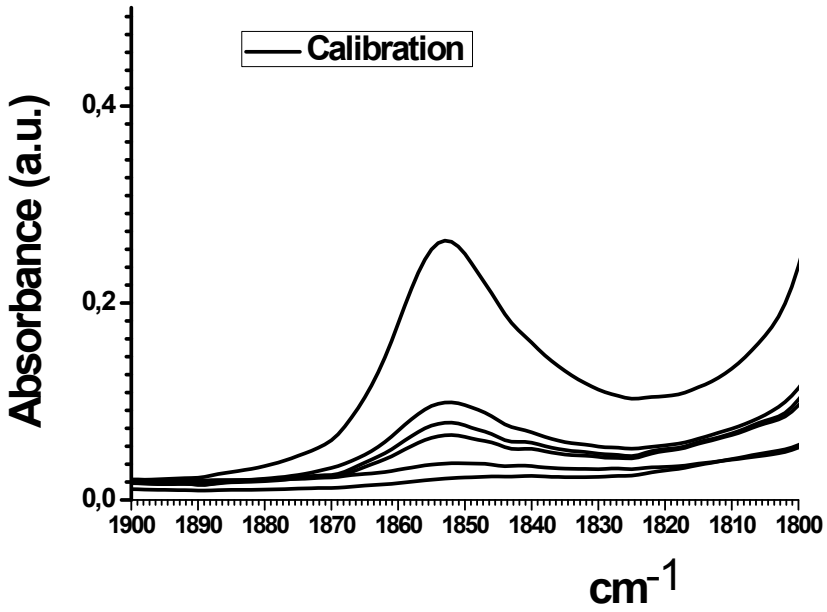
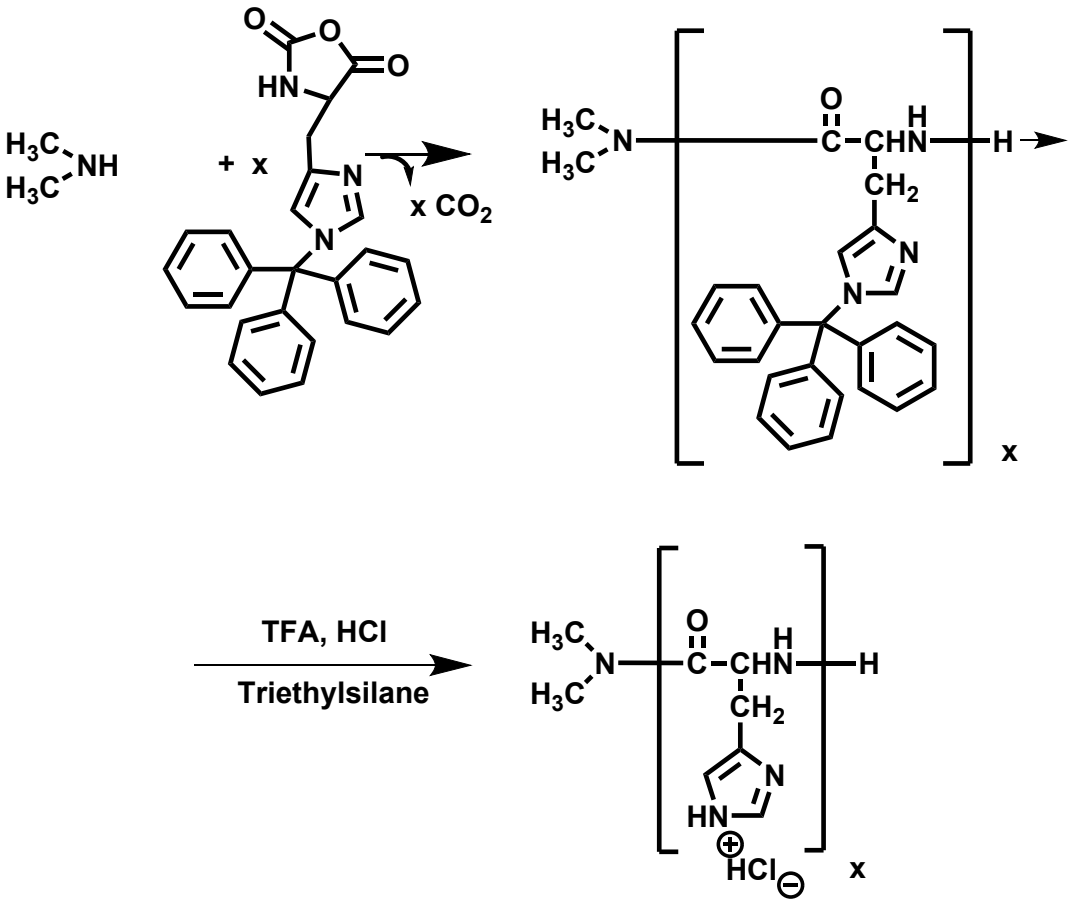


Figure F7. The calibration of HIS-NCA using the peak at 1850 cm^{-1} by FT-IR.

Polymer synthesis

Synthesis of linear poly(L-histidine) (PHIS) homopolymers, and diblock co- and terpolymers of PHIS.

Synthesis of linear PHIS homopolypeptides. The reactions used for the synthesis of PHIS homopolymers are shown in Scheme S5. The polymerizations were performed in a custom-made apparatus equipped with a high vacuum stopcock for periodic degassing of the solution. For the synthesis of PHIS 6K homopolymer, the apparatus was initially attached to the vacuum line through the ground joint and was evacuated and flame dried several times, and subsequently was transferred to the glove box and 1 g of *Trt*-HIS-NCA (2.36 mmol) were added. The apparatus was attached to the vacuum line and was pumped. Subsequently, 30 mL of highly pure DMF were distilled into the apparatus, the monomer was dissolved, and 0.0681 mmol of initiator dimethylamine (DMA) dissolved in 2 mL of pure DMF were added through the rupture of the break-seal of the ampoule containing the initiator in DMF. The solution was vigorously stirred and the consumption of the monomer was monitored by FT-IR through removal of an aliquot of the solution in the glove box. The solution was periodically pumped to remove the CO_2 produced from polymerization.



Scheme S5. Reactions used for the polymerization of HIS-NCA, to afford PHIS.

After completion of the polymerization, the polymers were precipitated in diethylether and dried under high vacuum. Then they were suspended in CH_2Cl_2 (20% w/v) and equal volume of trifluoroacetic acid (TFA) was added. The polymer was completely dissolved and was left to be deprotected for 1 hour at room temperature, and the solution became yellowish-light brown. Subsequently, an equimolar amount (with respect to the number of HIS monomeric units) of triethyl silane was added and the solution from yellowish-light brown turned colorless. The solution was poured in diethylether, and the white solid was filtered and dried. The white solid was dissolved in water at a pH~5 with addition of dilute HCl, and was

dialyzed against 2 liters of MilliQ water with pH~5 (adjusted with a dilute aqueous solution of HCl) for four times, and two times with pure MilliQ water. Finally, the polymer solution was freeze dried to yield PHIS as a white powder. Two linear PHIS homopolymers were synthesized one with molecular weight 6000 (PHIS 6K), similar to the PHIS block of the PEO-*b*-PHIS hybrid copolymers and terpolymers with PBLG and PLEU, described below, and one with a high molecular weight 35.0 x 10^3 g/mol (PHIS 35K). The obtained PHIS.HCl was 0.367 g (90 % yield). Mn=6.00 x 10^3 g/mol, Mw/Mn= 1.12. ^{1}H NMR (300 MHz, D_2O+DCl, δ in ppm): 2.55-2.95, (2H, CH-C$\underline{H_2}$-IM-*Trt*), 4.25-4.35 (1H, NH-C$\underline{H}$(CH_2-IM-*Trt*)-C=O), 6.9-7.0 (1H, C=C$\underline{H}$-N), 8.3-8.4 (1H, -N=C$\underline{H}$-N-), **IR** (thin film): 1645 cm^{-1} (vCO, s, amide I), (for trityl protected PHIS there are additionally two peaks, 700 cm^{-1}, 748 cm^{-1} (v =C-H out of plane bend, s)).

Synthesis of linear PEO-*b*-PHIS, PEO-*b*-P(HIS-*co*-BLG), PEO-*b*-P(HIS-*co*-LEU), poly(sarcosine-*b*-poly(L-histidine) (PSAR-*b*-PHIS)

Synthesis of PEO-*b*-PHIS, PEO-*b*-P(HIS-*co*-BLG), PEO-*b*-P(HIS-*co*-LEU) hybrid co- and terpolymers. One polymer of the PEO-*b*-PHIS type was synthesized along with a series of PEO-*b*-P(HIS-*co*-BLG) and a series of PEO-*b*-P(HIS-*co*-LEU) type diblock terpolymers. The total degree of polymerization of either PHIS or P(HIS-*co*-BLG) or P(HIS-*co*-LEU) remained constant and equal to 35, where the molar ratios of HIS/BLG or HIS/LEU varied and was equal to 90/10, 80/20, and 60/40. The molecular weight of PEO block was always the same, equal to 9.95 x 10^3 g/mol. The synthetic procedure for all diblock hybrid co- and terpolymers was similar.

The general procedure for the synthesis of PEO-*b*-PHIS will be presented. The polymerization was performed in a custom-made apparatus equipped with a high vacuum stopcock for periodic degassing of the solution and a magnetic stir bar covered with glass. The apparatus was initially attached to the vacuum line through the ground joint and was evacuated and flame dried several times and was then transferred to the glove box and 0.682 g of amine end-functionalized PEO (PEO-NH_2) with Mn=9.95 x 10^3 g/mol (0.0685 mmol of amines) was added. An apparatus similar to the one depicted in Scheme S6 but with one side ampoule E (instead of two depicted in this Scheme) was attached to the vacuum line and was pumped. Subsequently, 150 mL of highly dry benzene were distilled and the macroinitiator was dissolved. The solution was stirred for two hours and benzene was distilled off to dryness. Subsequently, 30 mL of highly pure DMF were distilled followed by dissolution of the macroinitiator. The apparatus was inserted again in the glove box, 1 g of HIS-NCA (2.36 mmol) (or a mixture of *Trt*-HIS-NCA and either BLG-NCA or LEU-NCA) were added in the side ampoule equipped with a ground joint and a constriction. The apparatus was attached to the vacuum line through the ground joint of the side ampoule containing the NCAs, evacuated, followed by distilling of 5 mL of highly pure DMF, and the apparatus was removed from the vacuum line trough heat sealing the constriction (B), and the *Trt*-HIS-NCA (or a mixture of *Trt*-HIS-NCA with LEU-NCA or BLG-NCA) were dissolved. The solution of the NCAs was added by rupture of the break-seal of the ampoule, and the solution was vigorously stirred. The consumption of the monomers was monitored by FT-IR through removal of an aliquot of the solution in the glove box. Periodically, the solution was pumped to remove the CO_2 produced from polymerization. After completion of the

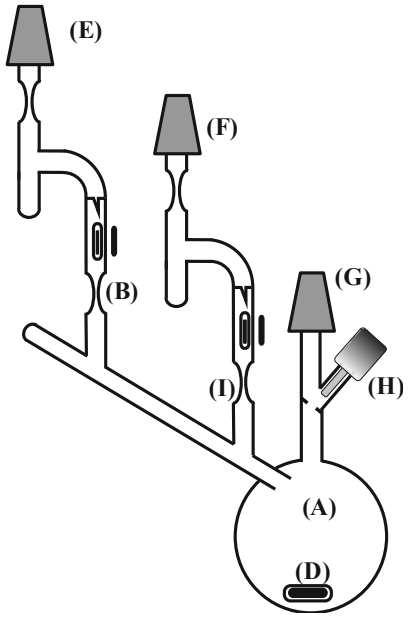
polymerization, the polymers were precipitated in diethylether and dried under high vacuum (1.50 g, 95 % yield).

The polymers were suspended in CH_2Cl_2 (20% w/v) and an equal volume of trifluoroacetic acid (TFA) was added. The polymers were completely dissolved and left to be deprotected for 1 hour at room temperature, and the solutions became yellowish-light brown. Subsequently, an equimolar amount of triethyl silane was added (with respect to the number of HIS monomeric units), and the solution from yellowish-light brown turned colorless. The solutions were poured in diethylether, and the white solids were filtered and dried. The white solids were subsequently dissolved in water at a pH~5 with addition of dilute HCl, and was dialyzed against 2 liters of MilliQ water with pH~5 (adjusted with a dilute aqueous solution of HCl) four times and two times in pure MilliQ water. Finally, the polymer solution was freeze dried to afford the corresponding polymer as a white powder. The obtained polymer was 0.92 g (86 % yield). Mn=15.80 x 10^3 g/mol, Mw/Mn= 1.11. ^{1}H NMR (300 MHz, D_2O+DCl, δ in ppm): 2.55-2.95 (2H, CH-C$\underline{H}_2$-IM), 3.25-3.5 (4H, -C$\underline{H}_2$-C$\underline{H}_2$-O) 4.25-4.35 (1H, NH-C$\underline{H}$(CH_2-IM)-C=O), 6.9-7.0 (1H, C=C$\underline{H}$-N), 8.3-8.4 (1H, -N=C$\underline{H}$-N-), IR (thin film): 1645 cm^{-1} (vCO, s, amide I), 1101 cm^{-1} (v-C-O-C, s), (for trityl protected PHIS there are additionally two peaks, 700 cm^{-1}, 748 cm^{-1} (v =C-H out of plane bend, s)).

For the PEO-*b*-P(HIS-*co*-BLG X), where x is 10, 20 and 40, typical yields were 85 %, for PEO-*b*-P(HIS-*co*-BLG 20) Mn=15.6 x 10^3 g/mol, Mw/Mn= 1.12. ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 1.6-2.0 (2H, C$\underline{H}_2$-CH_2-C=O benzyl ester) 2.25-2.5 (2H, CH_2-C$\underline{H}_2$-C=O benzyl ester), 2.90-3.10 (2H, CH-C$\underline{H}_2$-IM), 3.20-3.35 (4H, -C$\underline{H}_2$-C$\underline{H}_2$-O), 4.2-4.3 (1H, NH-C$\underline{H}$(CH_2-CH_2-*γ-benzyl* ester)) 4.90-5.1 (1H, NH-C$\underline{H}$(CH_2-IM)-C=O), 7.0-7.5

(5H, aromatic protons C=C-H), 8.0-9.1 (1H, -N=CH-N- and 1H, C=CH-N), IR (thin film): 1645 cm^{-1} (vCO, s, amide I), 1101 cm^{-1} (v-C-O-C, s), 700 cm^{-1}, 748 cm^{-1} (v =C-H out of plane bend, s), 1738 cm^{-1} (vCO, ester, s).

For the PEO-*b*-P(HIS-*co*-LEU X), where x is 10, 20 and 40, typical yields were 85 %, for PEO-*b*-P(HIS-*co*-LEU 20) Mn=16.0 x 10^3 g/mol, Mw/Mn= 1.09. ^{1}H NMR (300 MHz, CF_3COOD, δ in ppm): 0.6-0.9 (6H, -CH-(CH_3)$_2$), 1.2-1.7 (3H, -CH_2-CH-(CH_3)$_2$), 3.00-3.40 (2H, CH-CH_2-IM), 3.45-3.55 (4H, -CH_2-CH_2-O), 4.4-4.6 (1H, NH-CH(CH_2-CH-(CH_3)$_2$)) 4.80-5.1 (1H, NH-CH(CH_2-IM)-C=O), 7.2-7.5 (1H, C=CH-N), 8.40-8.60 (1H, -N=CH-N-), **IR** (thin film): 1645 cm^{-1} (vCO, s, amide I), 1101 cm^{-1} (v-C-O-C, s), (the *Trt*-protected has additional two peaks at 700 cm^{-1} and 748 cm^{-1} (v =C-H out of plane bend, s)).



Scheme S6. Apparatus used for the synthesis of the polymers. For PHIS homopolymers, instead of the side ground joints (F) and (E) an ampoule of the initiator was used, while for the diblock hybrid copolymers only one of the two side ampoules was used. For the PSAR-*b*-PHIS block copolypeptide, one of the two side ampoules shown was used and one ampoule contained the initiator.

Synthesis of the PSAR-*b*-PHIS diblock copolypeptides

The synthetic method followed for the PSAR-*b*-PHIS was similar to that described for the synthesis of the PEO-*b*-PHIS copolymer. For the synthesis of PSAR-*b*-PHIS 1, the general synthetic scheme is the following: DMA in DMF (0.0600 mmol in 2 mL of DMF) was utilized as the initiator, to polymerize first 420 mg of SAR-NCA (3.6 mmol) in 20 mL of highly purified DMF, followed by the addition of a DMF solution of HIS-NCA (370 mg, 0.867 mmol) after the completion of the polymerization of the first monomer. The deprotection of PHIS was performed in a similar way described for the PEO containing hybrids. The obtained polymer was 366 mg (89 % yield), and was characterized by SEC, lH NMR and FT-IR spectroscopy. Mn=4.80 x 103 g/mol, Mw/Mn= 1.11. ^{1}H NMR (Figure F8) (300 MHz, D2O+DCl, δ in ppm): 2.50-3.05 (5H, 2H from CH-CH2-IM and 3H from N-CH3 of PSAR), 3.75-4.25 (2H, N-CH2-C=O of PSAR) 4.25-4.35 (1H, NH-CH(CH2-IM)-C=O), 6.9-7.1 (1H, C=CH-N), 8.3-8.4 (1H, -N=CH-N-), IR (thin film): 1645 cm^{-1} (vCO, s, amide I), (for trityl protected PHIS there are additionally two peaks, 700 cm^{-1}, 748 cm^{-1} (v =C-H out of plane bend, s)).

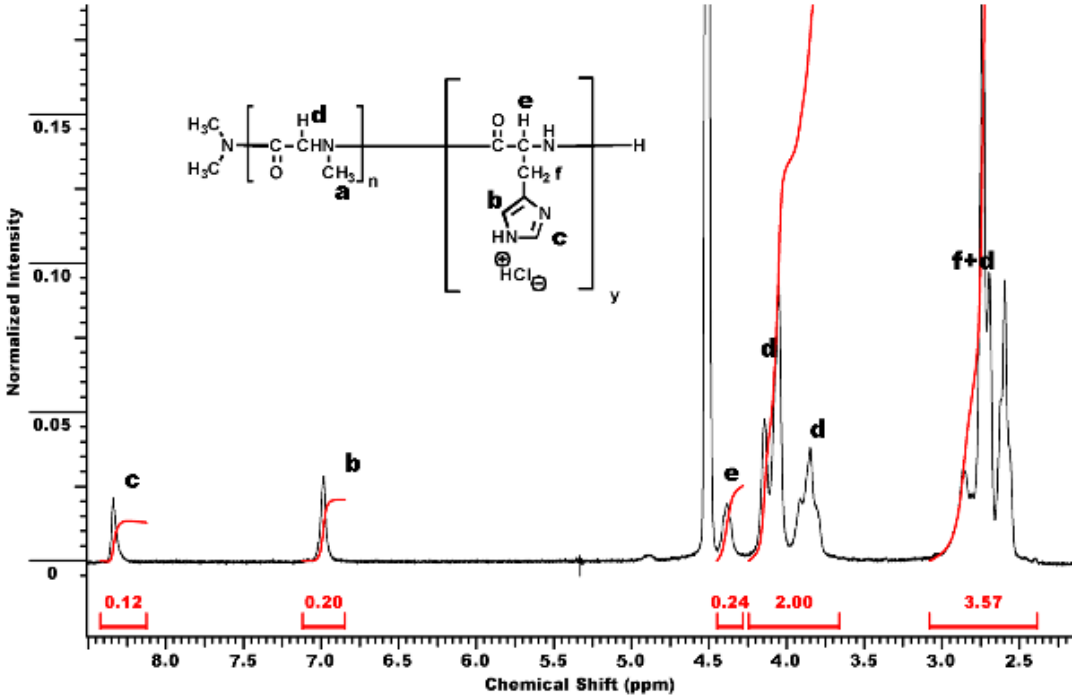


Figure F8. ^{1}H NMR spectrum of the PSAR-*b*-PHIS hybrid copolymer in D_2O+DCl.

Synthesis of linear PEO-*b*-PHIS-*b*-PLL triblock terpolymer and PEO-*b*-P(HIS-*co*-BLG 15)-*b*-PLL triblock quarterpolymer

The apparatus used for the synthesis of the triblock terpolymers is illustrated in Scheme S6. Each monomer was added through an ampoule formed *in situ* by dissolving the monomer with distilled DMF (E and F). Initial evacuation and drying of end amino-functionalized PEO is performed through ground joint G. The reactions used for the synthesis of PEO-*b*-PHIS-*b*-PLL are depicted in Scheme S7, where PLL is poly(L-lysine hydrochloride). The approach involved the synthesis of PEO-*b*-PHIS-NH_2 hybrid copolymer utilizing the strategy described in the previous section, i.e. 0.682 g of amine end-functionalized PEO (PEO-NH_2) with Mn=9.95 x 10^3 g/mol (0.0685 mmol of amines) was utilized as the macroinitiator, dried with 150 mL of dry benzene, and

1g of *Trt*-HIS-NCA (2.36 mmol) in 30 mL of DMF was added. A small aliquot was removed in the glove box and deprotected for SEC-TALLS characterization, followed by the addition of 0.408 g (1.5 mmol) BOCLL-NCA in a second ampoule. The ampoule containing the solution of BOCLL-NCA was prepared *in situ* on the apparatus like the ampoule of *Trt*-HIS-NCA, and was ruptured after the completion of the polymerization of *Trt*-HIS-NCA. After the completion of the polymerization the terpolymer was precipitated in diethyl ether and dried. It was then suspended in CH_2Cl_2 and the deprotection procedure was performed exactly as described for the PEO- containing diblocks in the previous section. The triblock terpolymer was characterized by SEC-TALLS, ^{1}H NMR and FT-IR spectroscopy. Mn=20.90 x 10^3 g/mol, Mw/Mn= 1.21. ^{1}H NMR (300 MHz, D_2O+DCl, δ in ppm):1.00-2.00 (6H, 2H CH-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-CH_2-NH_2.HCl), 2.50-2.65 (2H, CH-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-NH_2.HCl), 2.80-3.20 (2H, CH-C$\underline{H}_2$-IM), 3.25-3.5 (4H, -C$\underline{H}_2$-C$\underline{H}_2$-O) 4.00-4.25 (1H, NH-C$\underline{H}$(CH_2-CH2-CH2-CH2-NH2)-C=O), 4.45-4.55 (1H, NH-C$\underline{H}$(CH_2-IM)-C=O), 7.3-7.5 (1H, C=C$\underline{H}$-N), 8.3-8.4 (1H, -N=C$\underline{H}$-N-), IR (thin film): 1645 cm^{-1} (vCO, s, amide I), 1101 cm^{-1} (v-C-O-C, s), (for trityl protected PHIS there are additionally two peaks, 700 cm^{-1}, 748 cm^{-1} (v =C-H out of plane bend, s)).

The synthetic approach of the quarterpolymer was similar to that described for the PEO-*b*-PHIS-*b*-PLL triblock terpolymer, with the only difference being that 15 % of the monomeric units of the HIS-NCA were replaced by BLG-NCA (instead of 1 g of *Trt*-His-NCA 0,850 g *Trt*-His-NCA (2.01 mmol) and 0.0931 g (0.35 mmol) BLG-NCA) was added, therefore resulting in the copolymerization of these NCAs, and to the formation of a middle block of the type P(HIS-*co*-BLG) instead of PHIS. The reactions

used are shown in Figure F9. The deprotection was the same described for the PEO containing diblock copolymers. The apparatus utilized for the synthesis of the triblocks is shown in Scheme S6. The triblock quarterpolymer was characterized by SEC-TALLS, NMR and IR spectroscopy. Mn=20.85 x 10^3 g/mol, Mw/Mn= 1.20. ^{1}H NMR (300 MHz, DMSO-d6+CF_3COOD, δ in ppm): 1.00-2.00 (8H, 6H CH-C<u>H</u>$_2$-C<u>H</u>$_2$-C<u>H</u>$_2$-CH_2-NH_2.HCl and 2H CH-C<u>H</u>$_2$-CH_2-benzyl ester), 2.55-2.75 (4H, 2H CH-CH_2-CH_2-CH_2-C<u>H</u>$_2$-NH_2.HCl, 2H CH-CH_2-C<u>H</u>$_2$-benzyl ester), 2.80-3.20 (2H, CH-C<u>H</u>$_2$-IM), 3.25-3.5 (4H, -C<u>H</u>$_2$-C<u>H</u>$_2$-O) 3.80-4.50 (2H, 1H NH-C<u>H</u>(CH_2-CH_2-CH_2-CH_2-NH_2)-C=O and 1H C<u>H</u>-CH_2-CH_2-benzyl ester), 4.55-4.75 (1H, NH-C<u>H</u>(CH_2-IM)-C=O), 4.90-5.10 (2H, CH_2-COO-C<u>H</u>$_2$-Ph), 7.2-7.5 (6H, 1H C=C<u>H</u>-N and 5H of Phenyl group), 8.3-8.4 (1H, -N=C<u>H</u>-N-), IR (thin film): 1645 cm^{-1} (vCO, s, amide I), 1101 cm^{-1} (v-C-O-C, s), (for trityl protected PHIS there are additionally two peaks, 700 cm^{-1}, 748 cm^{-1} (v =C-H out of plane bend, s)), 1738 cm^{-1} (vCO, ester, s).

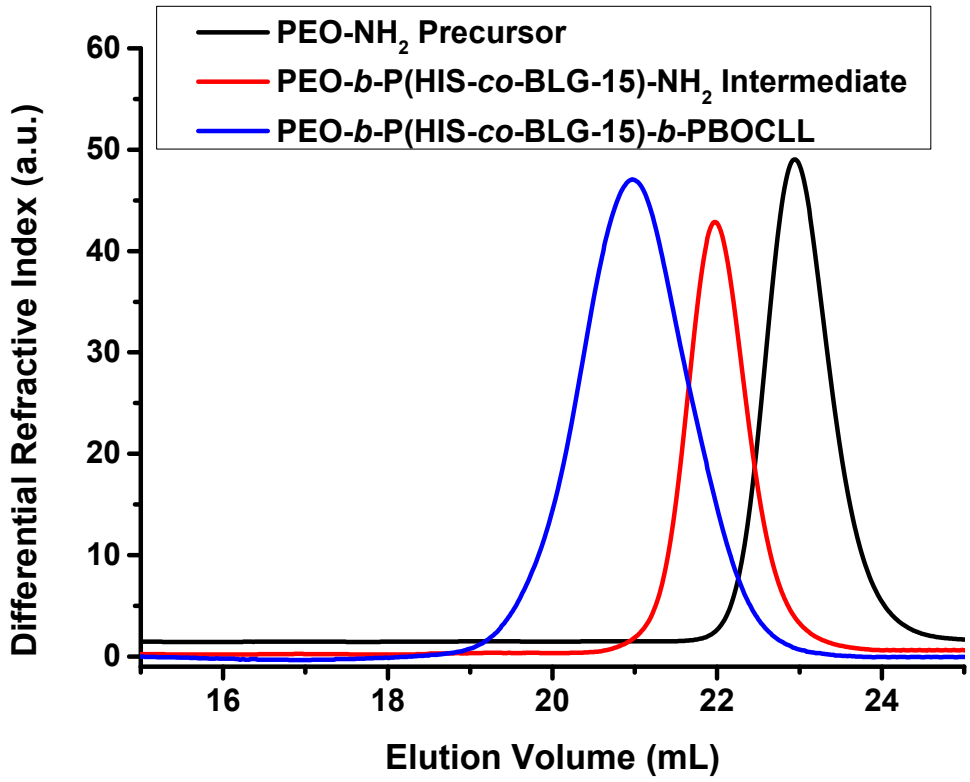
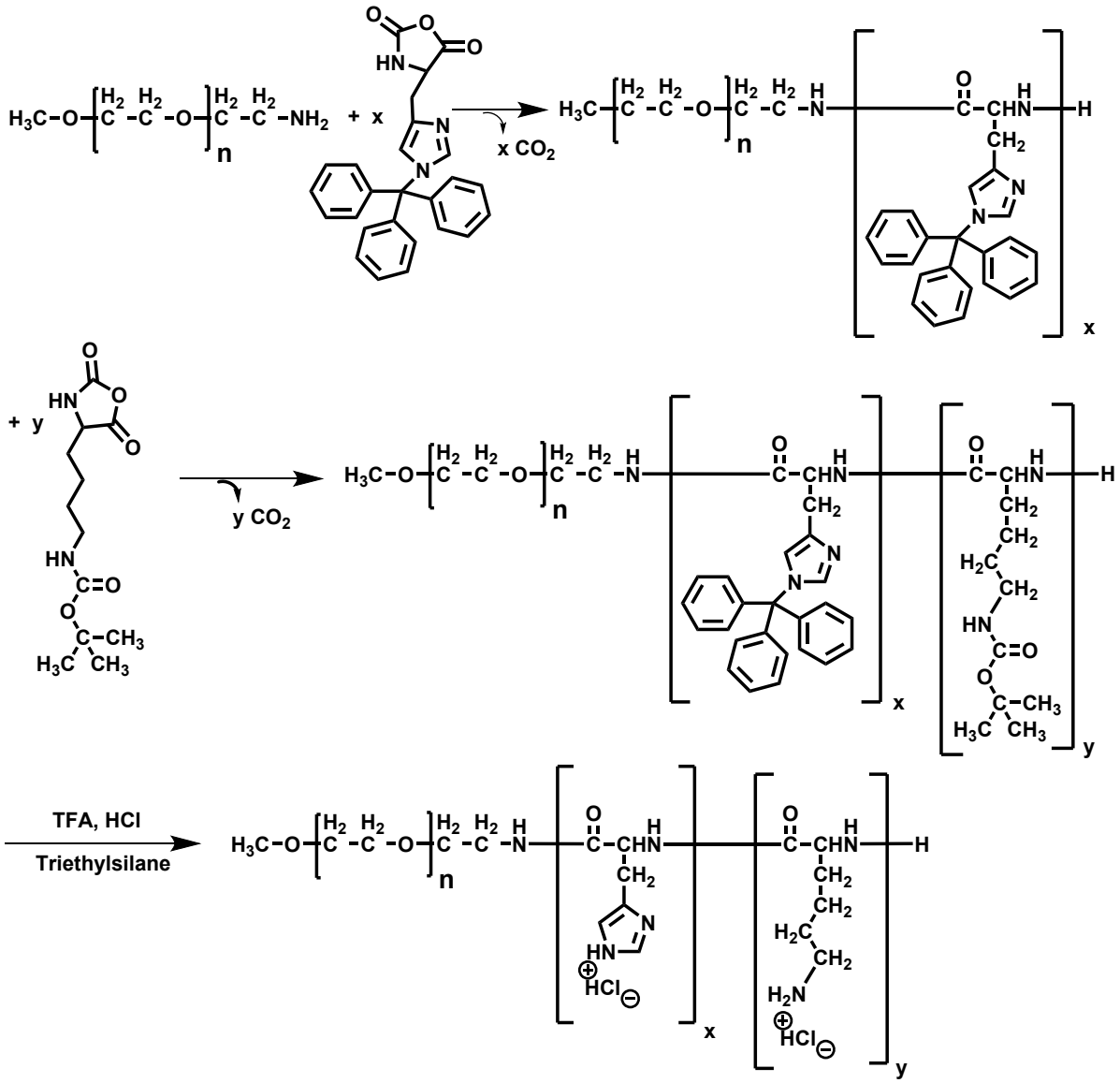


Figure F9. SEC eluograms of the synthesis of the PEO-*b*-P(HIS-*co*-BLG-15)-*b*-PLL hybrid copolymer.



Scheme S7. Reaction scheme for the synthesis of PEO-*b*-PHIS-*b*-PLL triblock terpolymer.

Synthesis of block-graft PBLG-*b*-(PLL-*g*-P(*Trt*)HIS) terpolypeptide

The synthetic approach first involved the synthesis of the backbone, i.e. the synthesis of PBLG-*b*-PBOCLL diblock copolypeptide. The copolypeptide was synthesized using DMA as the initiator and (0,068 mmol dissolved in two mL of DMF) the sequential addition of BLG-NCA (1.80 g, 6.84 mmol) followed, after the

completion of polymerization, by the addition of BOCLL-NCA (0.190 g, 0.70 mmol). The copolypeptide was precipitated in diethyl ether, and the white polymer was dissolved in DCM (20% w/v) followed by the addition of equal volume of TFA. The dissolved polymer was left to react for one hour, then the solvents were evaporated, followed by the addition of an appropriate amount of MilliQ water (10 % polymer in water w/w), and the solution was dialyzed two times against a solution of 2 liters MilliQ water with a few drops of concentrated HCl to reach a pH=5. The dialysis bag was then placed twice in 2 liters of pure MilliQ water and then in MilliQ water that the pH was brought at pH=9.0 and once in pure MilliQ water to remove the salts, and was subsequently freeze-dried, to afford 1.54 g. Yield: 84 %.

1.00 g (0.434 mmol of primary amines) of the resulting PBLG-*b*-PLL diblock copolypeptide was dissolved in 30 mL of purified DMF, followed by the addition of 0.73 g (1.73 mmol) of HIS-NCA was added as a solution in 5 mL of DMF. The polymerization was left to completion for six days, followed by precipitation in diethyl ether, resulting to 1.57 g (95 % yield). The polymer was characterized by SEC-TALLS, ^{1}H NMR and FT-IR spectroscopy. Mn=38.4 x 10^3 g/mol, Mw/Mn= 1.12. ^{1}H NMR (300 MHz, DMSO-d6+CF_3COOD, δ in ppm):0.90-2.30 (8H, 6H CH-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-CH_2-NH_2.HCl and 2H CH-C$\underline{H}_2$-CH_2-benzyl ester), 2.55-2.75 (4H, 2H CH-CH_2-CH_2-CH_2-C$\underline{H}_2$-NH_2.HCl, 2H CH-CH_2-C$\underline{H}_2$-CO-benzyl ester), 2.80-3.20 (2H, CH-C$\underline{H}_2$-IM), 3.50-4.80 (3H, 1H NH-C$\underline{H}$(CH_2-CH_2-CH_2-CH_2-NH_2)-C=O, 1H C$\underline{H}$-CH_2-CH_2-benzyl ester and 1H NH-C$\underline{H}$(CH_2-IM)-C=O), 4.90-5.10 (2H, CH_2-COO-C$\underline{H}_2$-Ph), 6.8-7.5 (21H, 1H C=C$\underline{H}$-N, 5H of Phenyl group of PBLG and 15H from trityl group), 8.5-9.0 (1H, -N=C$\underline{H}$-N-), IR (thin film): 1645 cm^{-1} (vCO, s, amide I), 1101 cm^{-1} (v-C-O-C, s), (for trityl protected PHIS

there are additionally two peaks, 700 cm^{-1}, 748 cm^{-1} (v =C-H out of plane bend, s)), 1738 cm^{-1} (vCO, ester, s).

Turbidity measurements

The pK_a of PHIS 6K along with PHIS 35K was obtained from the titration of an aqueous solution of the homopolypeptide and turbidity measurements. The homopolypeptide (0.010 g) were dissolved in 3 mL of MilliQ water, and was left to be completely dissolved overnight. Three drops of HCl 1N solution were added to reach a pH=2.5, in order for all PHIS monomeric units to be protonated and dissolved. The solution was titrated with an aqueous solution 0.1N NaOH in MilliQ water and the absorption of the solution was monitored at 500 nm. When HIS starts to become deprotonated, it becomes hydrophobic, leading to aggregation and precipitation. Measurements were carried out with a very sensitive spectrometer, and the NaOH solution used was concentrated in order to avoid significant dilution during titration. The polymer concentration was normalized to the added volume of NaOH solution. At about pH=5.5, the solutions started to absorb until pH=10.5.

Influence of the LEU and BLG composition on the pK_a of PHIS

In order to examine the influence of a hydrophobic amino acid randomly distributed along the PHIS chain, 0.03 g of PHIS was dissolved in 2.0 mL of MilliQ water, and was left overnight to be completely dissolved. Depending on the composition of pure PHIS at every polymer, an appropriate amount of the polymer was weighed in order to always have the same PHIS composition, i.e. 0.030 g in 2.0

mL of MilliQ water. The next day, a few drops of HCl 1N were added to bring the pH=2.00, and the solution was titrated with an aqueous 0.01N NaOH solution. The pH at the end point was monitored as a function of the hydrophobic amino acid. The titration was performed for PHIS 6K and PHIS 35K homopolypeptides, along with PEO-*b*-PHIS, PEO-*b*-PHIS, PEO-*b*-P(HIS-*co*-BLG-X) and PEO-*b*-P(HIS-*co*-LEU-X) diblock terpolymers.

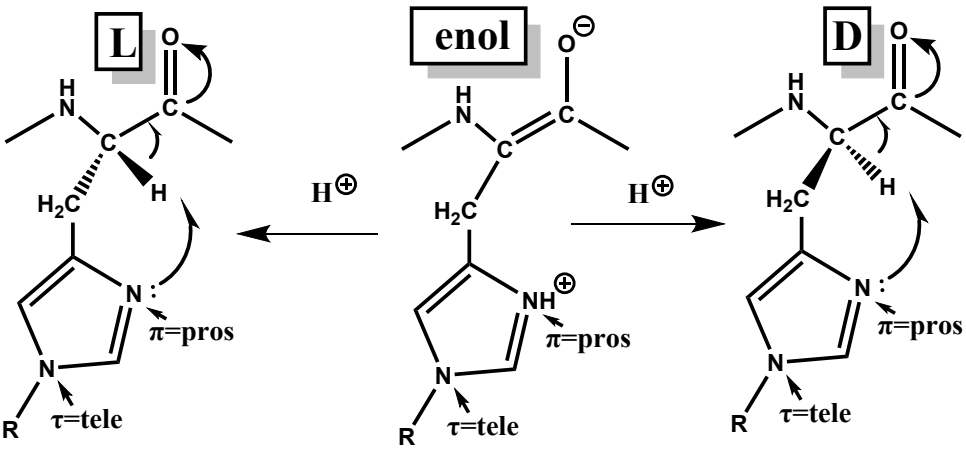
Influence of the pH on the aggregation of diblock co- and terpolymers along with triblock ter-and quarterpolymers in water, obtained with dynamic light scattering (DLS)

The pH sensitivity of the aggregates containing PHIS was examined at physiological pH=7.4 and at pH of the late endosomes equal to 5.0. The polymers examined were PEO-*b*-PHIS diblock copolymer, PEO-*b*-P(HIS-*co*-BLG), PEO-*b*-P(HIS-*co*-LEU) diblock terpolymers along with PEO-*b*-PHIS-*b*-PLL triblock terpolymer and PEO-*b*-P(HIS-*co*-BLG 15)-*b*-PLL triblock quarterpolymer. Concentrations utilized were ranged between 2.0 -3.0 x 10^{-3} g/mL.

Circular dichroism

A dilute solution of the samples in a concentration of the polypeptide block 1x10^{-4} g/ml was measured at various pH using a 0.1 Quartz Suprasil cell. The pH was initially established at 3.25 by adding a dilute solution of HCl in the MilliQ aqueous solution of PHIS. The pH was changed using a concentrated 1N NaOH solution by the addition of a few microliters each time, in order avoid significant change of the

polymer concentration. The measurements were conducted at 3, 20, 37, 50 and 80 ^{o}C.



Scheme S8. Racemization of Histidine during the cleavage of the protecting group.

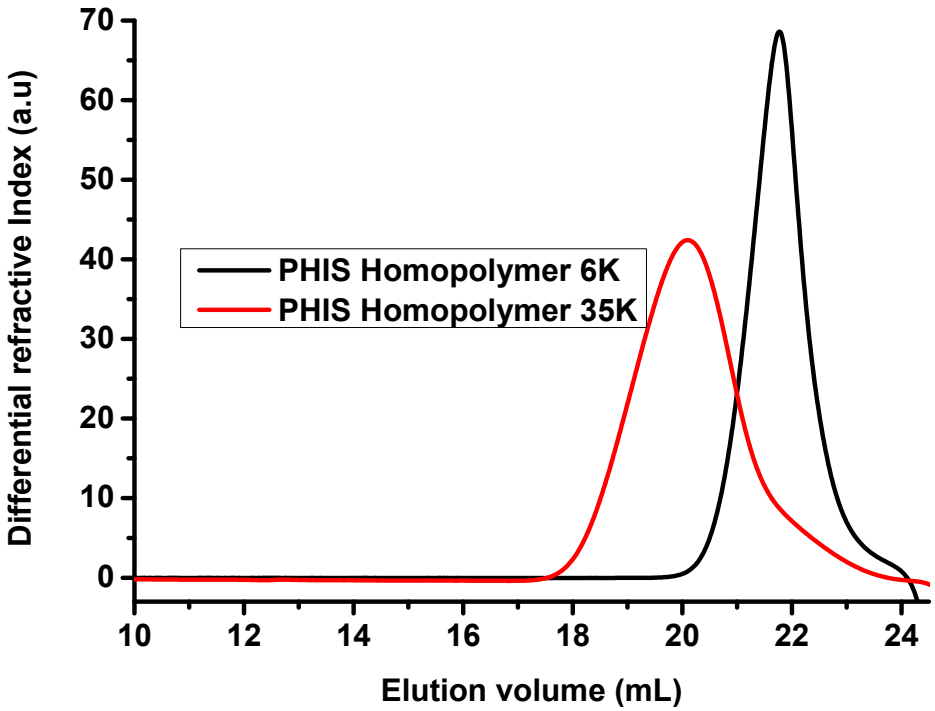


Figure F10. Size exclusion eluograms of the PHIS 6K (black line) and PHIS 35K (red line).

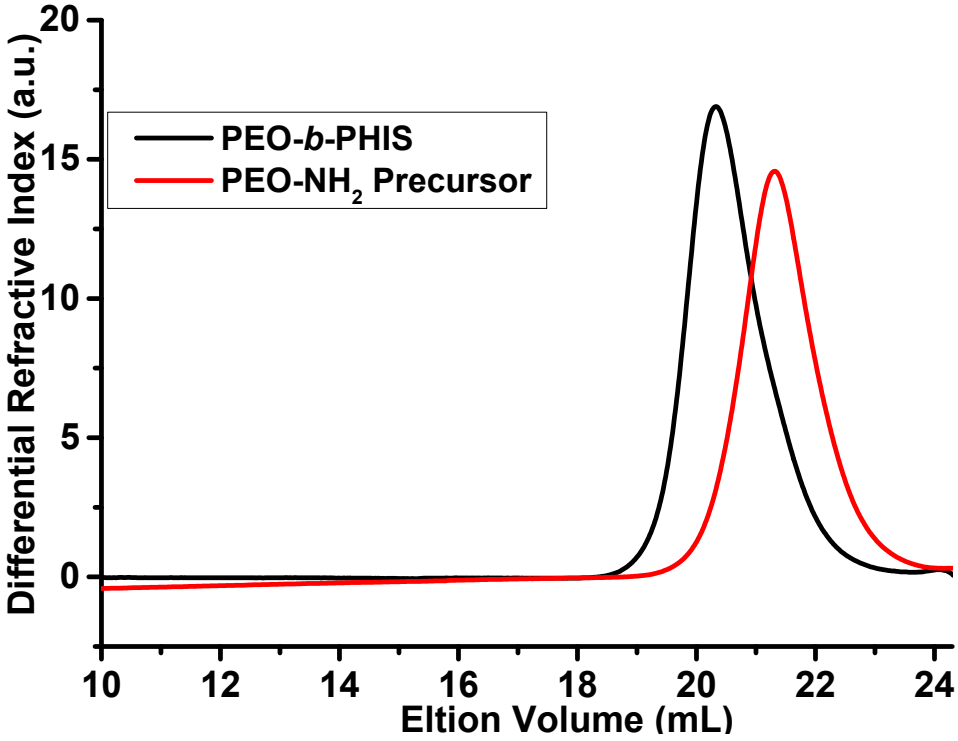


Figure F11. SEC eluogram of the PEO-*b*-PHIS hybrid diblock copolymer.

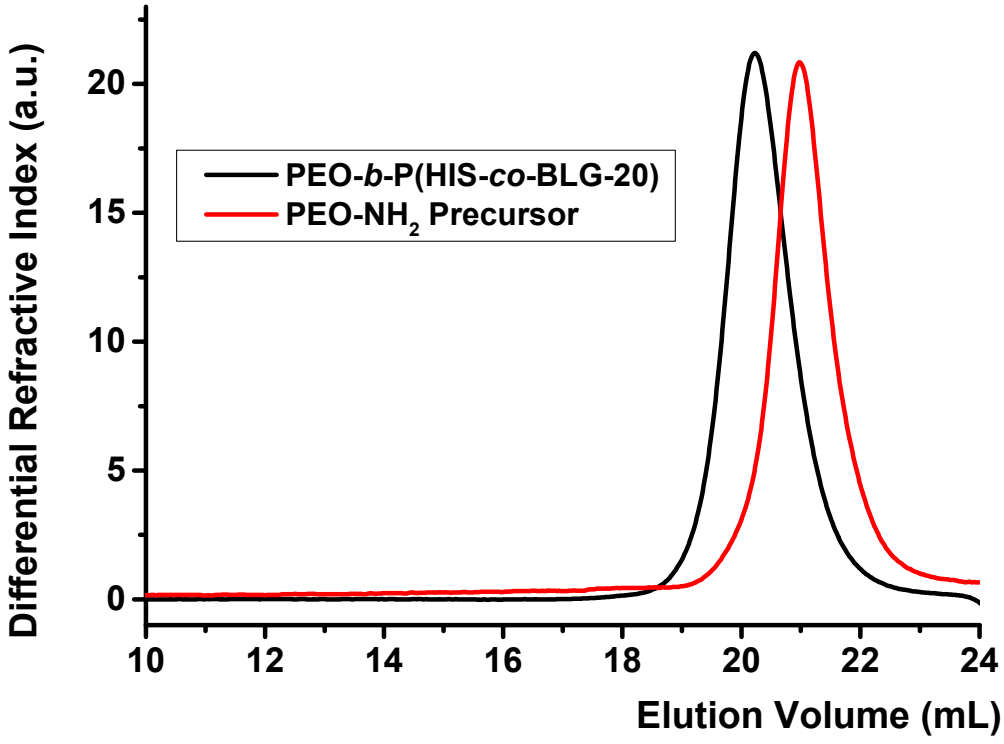


Figure F12. SEC eluogram of the PEO-*b*-P(HIS-*co*-BLG-20) hybrid diblock copolymer.

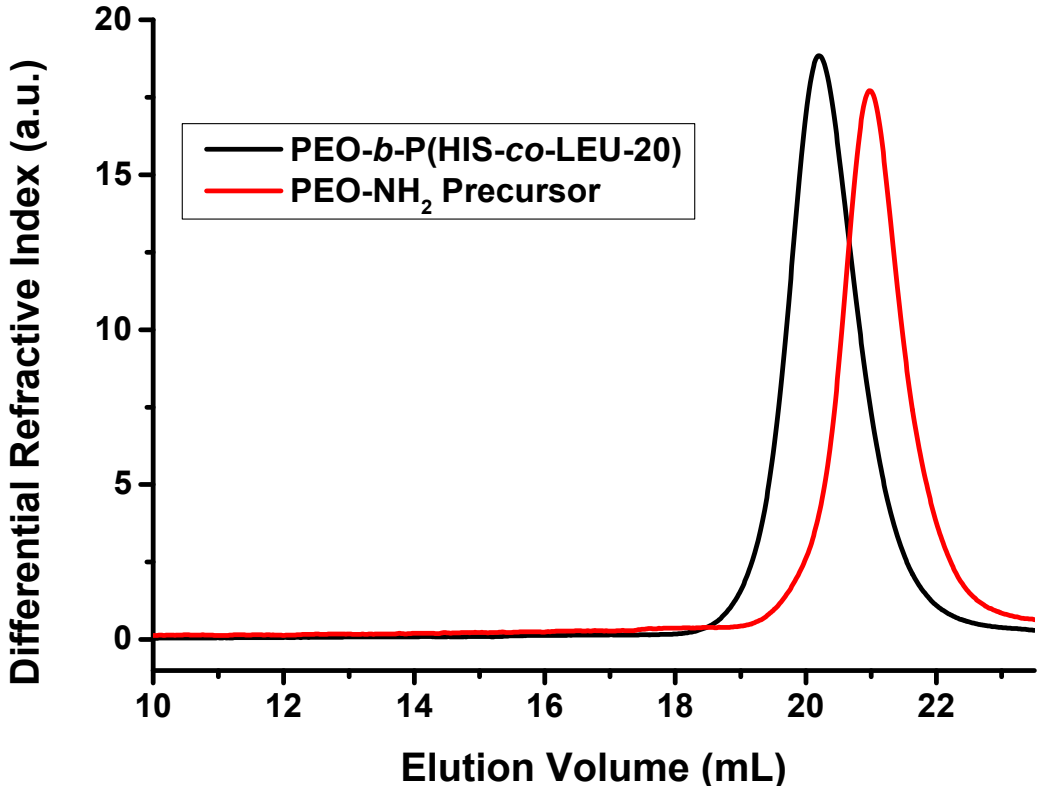


Figure F13. SEC eluogram of the PEO-*b*-P(HIS-*co*-LEU-20) hybrid diblock copolymer.

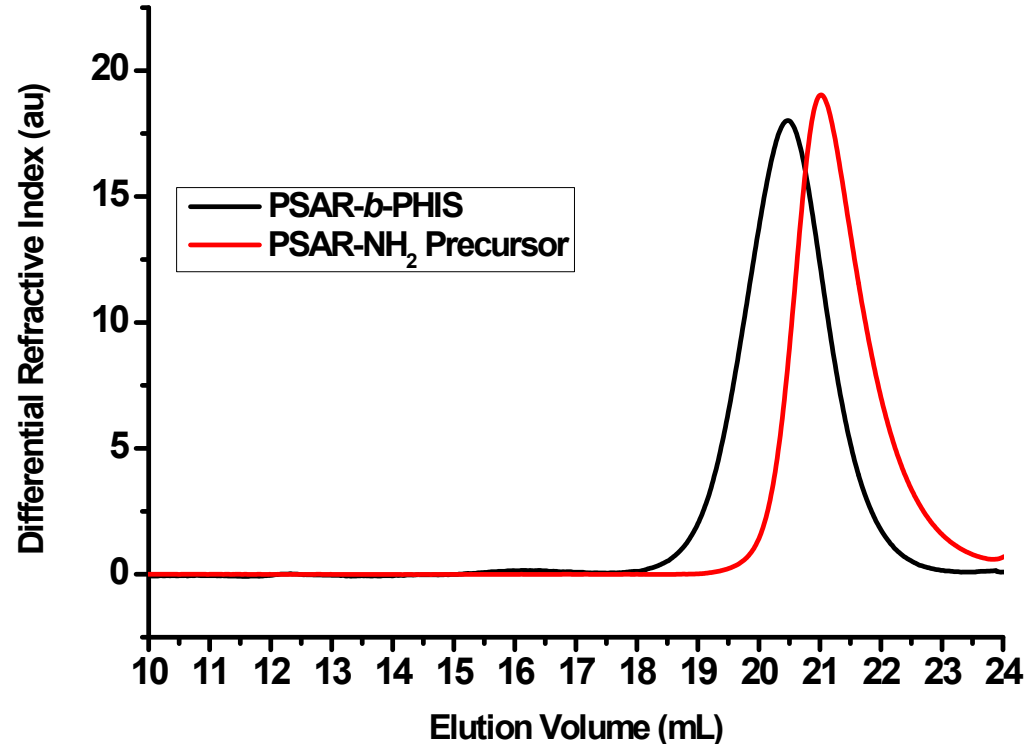


Figure F14. SEC eluogram of the synthesis of the PSAR-*b*-PHIS.

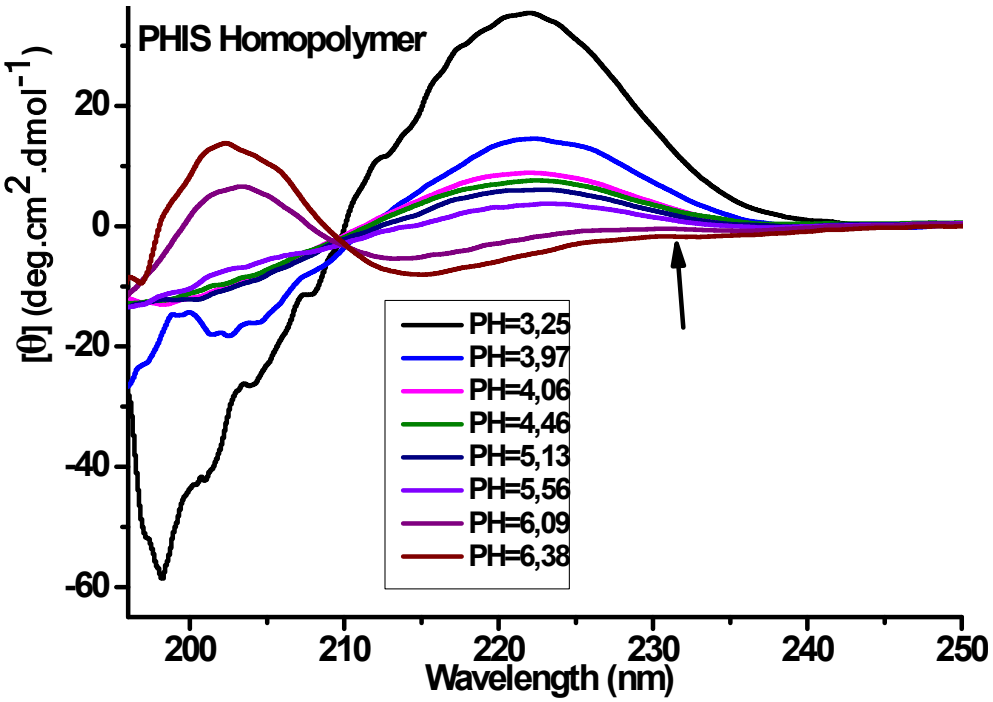
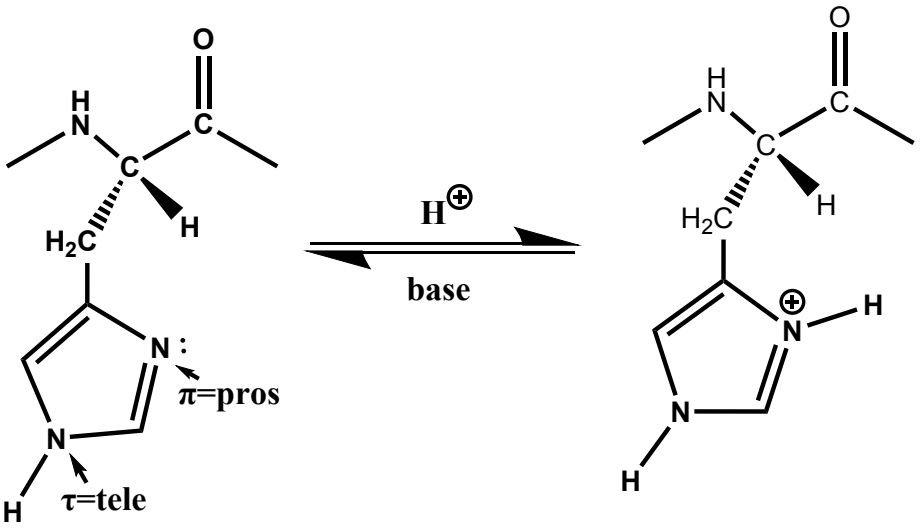


Figure F15. CD of PHIS-6K homopolypeptide as a function of pH.



Scheme S9. Protonation of PHIS

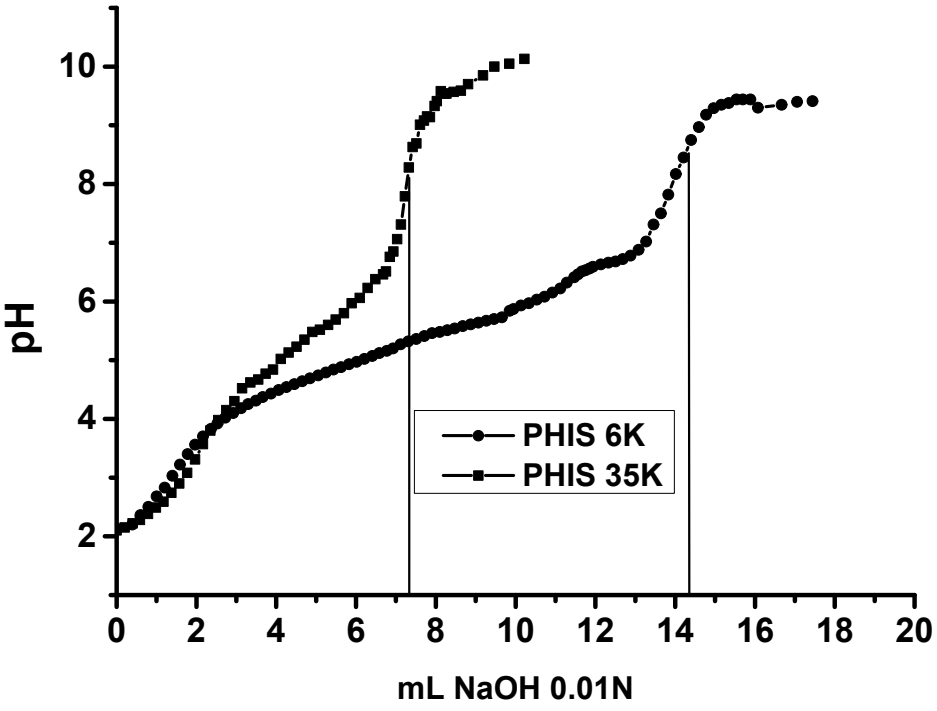


Figure F16. Titration of PHIS homopolymers with different amounts, 10.0 mg of PHIS 35K and 19.7 mg of PHIS 6K.

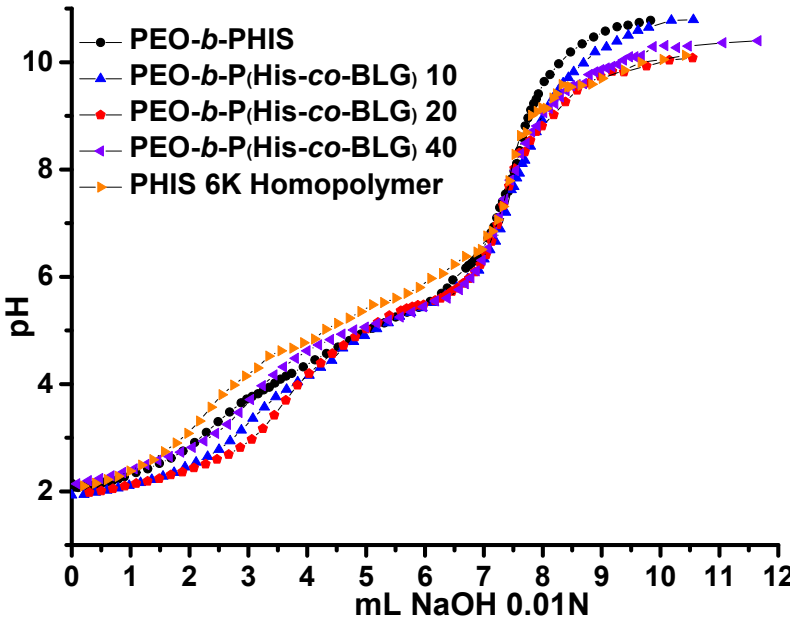


Figure F17. Titration curves of PEO-*b*-PHIS, and PEO-*b*-P(HIS-*co*-BLG-X) with X = 10, 20 and 40.

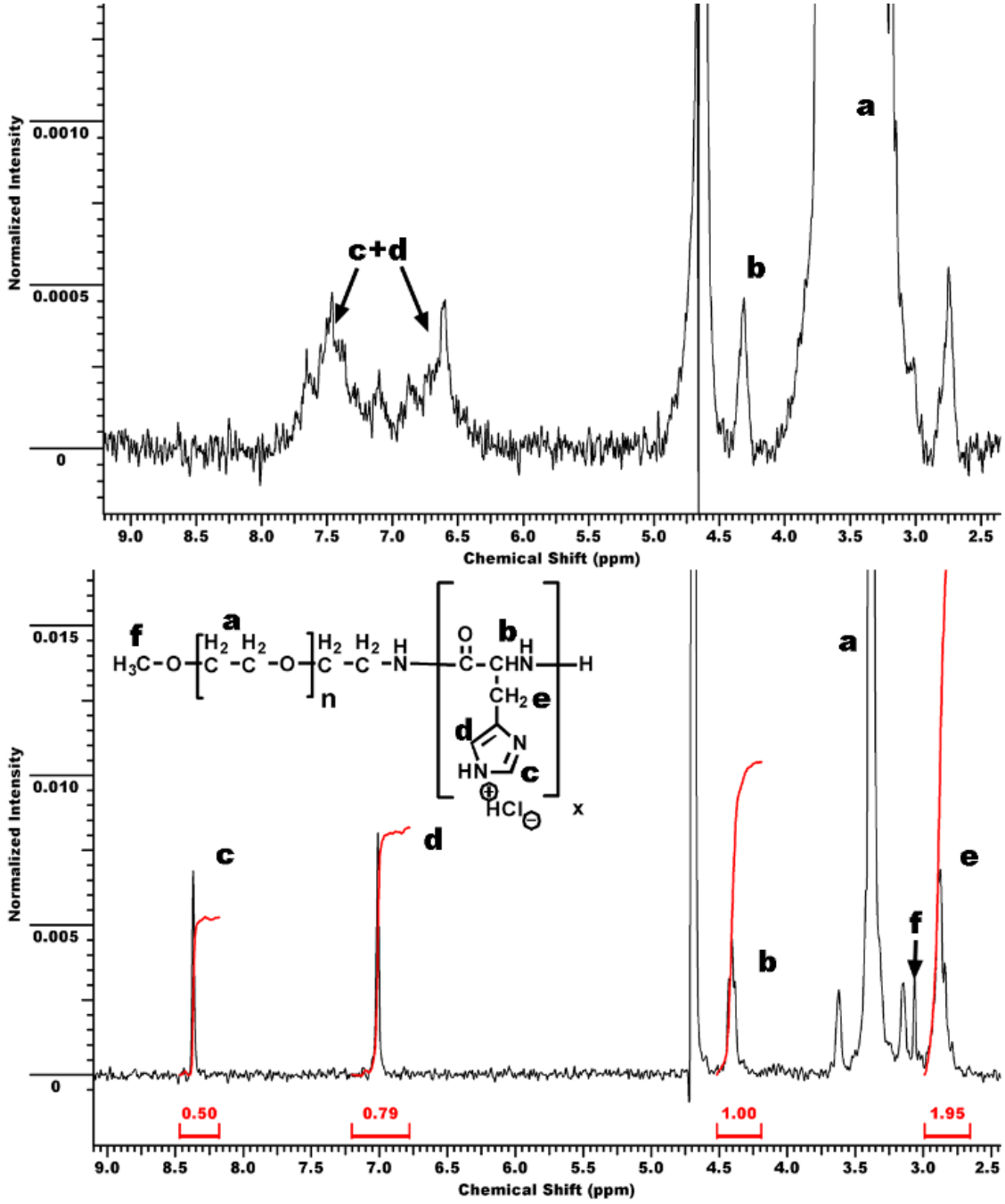


Figure F18. Aggregated PHIS in the diblock PEO-*b*-PHIS at neutral pH (top) and solvated PHIS in the same diblock at low pH.

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