Supplementary Information

Single-molecule force spectroscopy shows that side chain interactions govern the mechanochemical response of polypeptide α-helices and prevent the formation of β-sheets

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1. Experimental details

1.1. Synthesis and Characterization of PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-(2-pyridyl disulphide)

1.1.1. Synthesis of PEG_{114}-b-poly(N^{ε}-trifluoroacetyl-L-lysine)$_{134}$-NH_2 by ROP of N^{ε}-TFA-L-Lys-NCA

The primary amine used to initiate the polymerization of N^{ε}-TFA-*L*-Lys-NCA was PEG_{114}-NH_2 **(2)** which was synthesized from PEG_{114}-OH in two steps, PEG_{114}-SO_3CH_3 **(1)** being an intermediate compound (Figure S1). The synthesis was adapted from ref. 1.

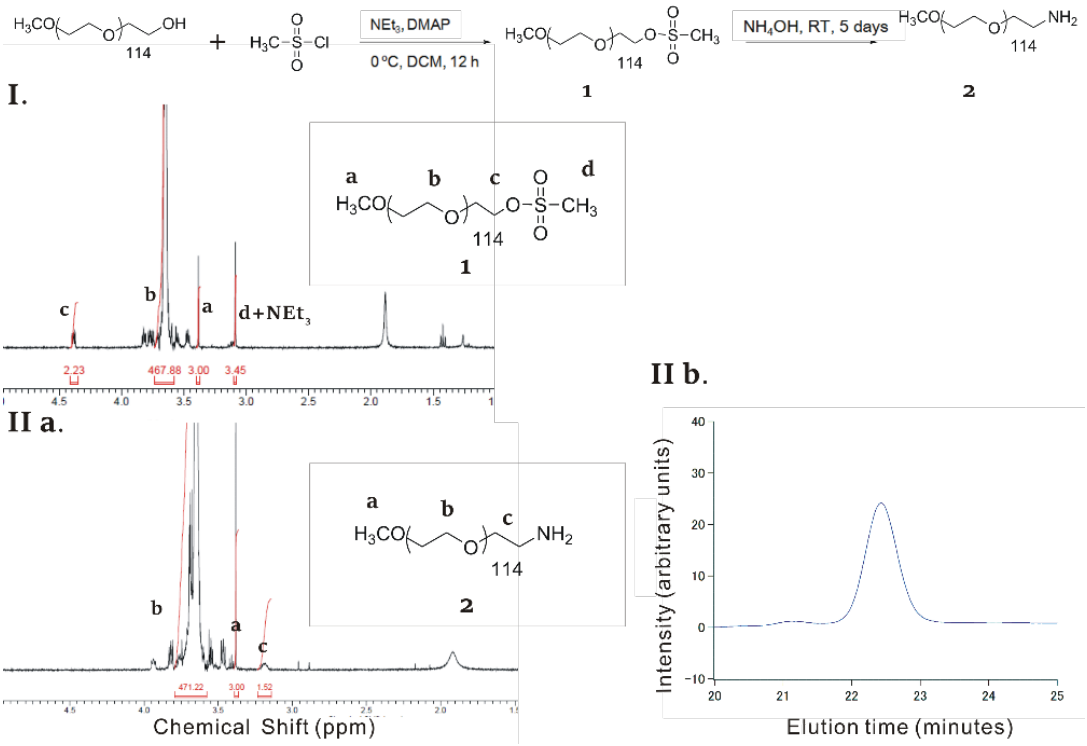

Figure S1. Synthesis of PEG_{114}-NH_2 from PEG-OH with PEG-SO_3CH_3 as an intermediate compound. I.) ^{1}H NMR of PEG-SO_3CH_3 in $CDCl_3$ (Bruker 400MHz). Full functionalization of the chain end was confirmed by comparing the integration of the protons of the methyl group labelled as **a** (CH_3O, 3.38 ppm) with that of the ethyl protons beside the mesylate **c**, ($CH_2OS(O_2)CH_3$, 4.38 ppm). II a.) ^{1}H NMR of PEG-NH_2 in $CDCl_3$ (Bruker 400MHz). Full functionalization of the chain end was confirmed by ^{1}H NMR by comparing the integration of the protons of the methyl end-group labelled as **a** (CH_3O, 3.38 ppm) with that of the ethyl protons beside the amine end-function **c**, (CH_2NH_2, 3.19 ppm). II b.) SEC of PEG-NH_2 in DMF containing 1g·L^{-1} LiBr, $Đ\frac{Mw}{Mn}$=1.02.

After PEG_{114}-NH_2 **(2)** was successfully obtained, it was used for subsequent ROP of N^{ε}-TFA-*L*-Lys-NCA (Figure S2). The temperature of the reaction was lowered to 5°C and the combined reactants formed a highly viscous solution as the targeted degree of polymerization was relatively high and the PEG initiator was also relatively long. After 5 days, by raising the temperature, slightly good solubility was obtained without adding extra DMF to the reaction vessel, as it has to be partially removed prior to precipitation. The degree of polymerization was controlled by adjusting the monomer/initiator ratio.

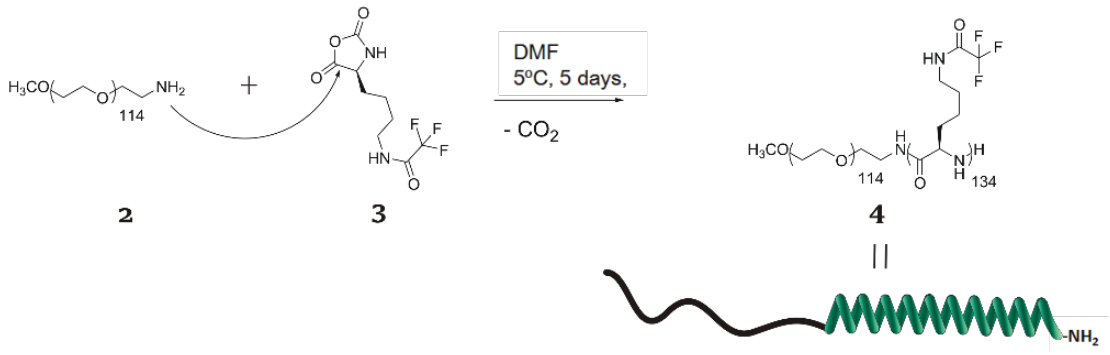

Figure S2. Initiation and growth of PEG-NH_2 initiated ROP of N^{ε}-TFA-*L*-Lys-NCA

The resulting degree of polymerization (DP ≈ 134) was determined by comparing the integration of the peak corresponding to the CH_2 signal nearest to the trifluoroacetyl group labelled as **(d)** ($C\mathbf{H_2}NHCOCF_3$, 3.13 ppm) with that of the integration of the peak corresponding to the PEG_{114} ($C\mathbf{H_2}C\mathbf{H_2}O$, 3.51 ppm) labelled as **(a)** (Figure S3). A small secondary peak can be seen in the SEC trace, which was thought to be an effect of secondary structures in the DMF solvent.[2]

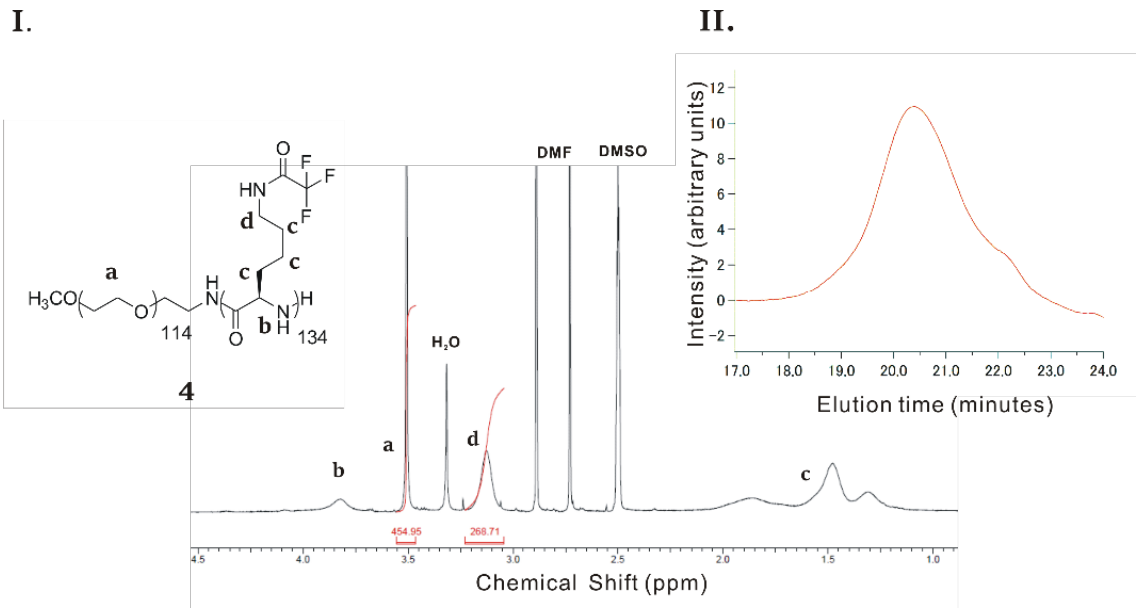

Figure S3. ^{1}H NMR spectrum and SEC trace of the resulting PEG_{114}-*b*-poly(N^{ε}-trifluoroacetyl-*L*-lysine)$_{134}$-NH_2. II a.) ^{1}H NMR of PEG_{114}-*b*-poly(N^{ε} trifluoroacetyl-*L*-lysine)$_{134}$-NH_2 in DMSO-d_6 (Bruker 400MHz). II b.) SEC of PEG_{114}-*b*-poly(N^{ε}-trifluoroacetyl -*L*-lysine) $_{134}$-NH_2 in DMF containing 1g·L^{-1} LiBr , dispersity $Đ=\frac{Mw}{Mn}$=1.16; 80 °C.

1.1.2. Synthesis of PEG_{114}-b-poly(N^{ε}-trifluoroacetyl-L-lysine)$_{134}$-(2-pyridyl disulphide) by coupling reaction of N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) to the N-terminal amino group

Amine-coupling reaction by means of an *N*-hydroxysuccinimide (NHS) ester was used with *N*-

succinimidyl 3-(2-pyridyldithio)propionate (SPDP) to couple a 2-pyridyl disulphide group to the terminal amine of PEG_{114}-*b*-poly(N^{ε}-trifluoroacetyl-*L*-lysine)$_{134}$-NH_2 **(4)** (Figure S4). After reaction, the disulphide moiety was not reduced to a thiol as disulphides tend to be more stable and they are similarly capable forming Au-S bonds with a gold surface. The PEG_{114}-*b*-poly(N^{ε}-trifluoroacetyl-*L*-lysine)$_{134}$-(2-pyridyl disulphide) **(5)** was then deprotected by adding KOH to cleave the trifluoroacetyl group, resulting in the final product PEG_{114}-*b*-(poly-*L*-lysine)$_{134}$-(2-pyridyl disulphide) **(6)**.

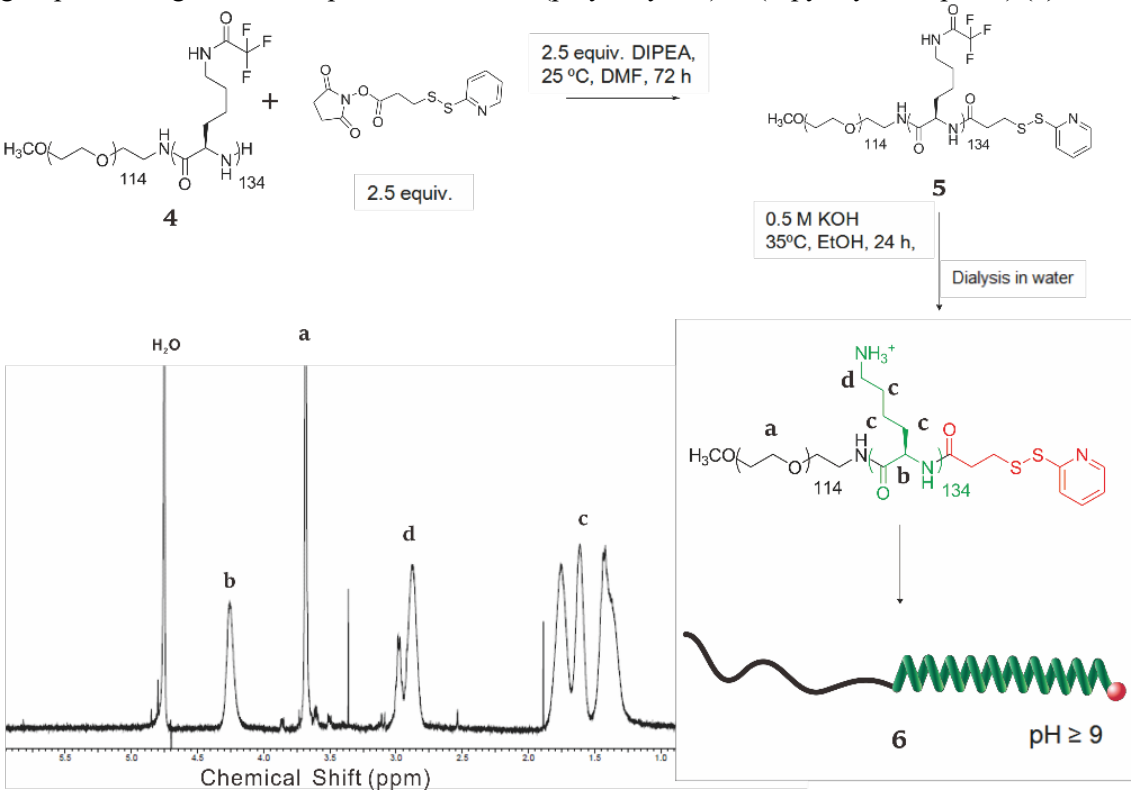

Figure S4. Coupling reaction of *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) with PEG_{114}-*b*-poly(N^{ε}-trifluoroacetyl-*L*-lysine)$_{134}$-NH_2, and subsequent deprotection. ^{1}H NMR of PEG_{114}-*b*-(poly-*L*-lysine)$_{134}$-(2-pyridyl disulphide) in D_2O (Bruker 400MHz). The schematic representation depicts the deprotonated lysine side chain at high pH allowing the polypeptide segment to fold into a α-helix. In the conditions utilized for the synthesis, and purification the molecule would adopt a random coil conformation as also indicated by the position of the ε–CH_2 peak **(d)** ≈ 3.0 ppm

The ^{1}H NMR spectrum of PEG_{114}-*b*-poly(N^{ε}-trifluoroacetyl-*L*-lysine)$_{134}$-(2-pyridyl disulphide) **(5)** is not shown as the signal of the 2-pyridyl disulphide molecule is comparatively small to the rest of the molecule and cannot be seen clearly in the ^{1}H NMR spectrum. We acknowledge this significant weakness in the characterization, however for the purpose of this work even if not all molecules were not coupled to the (2-pyridyl disulphide) they would simply not be immobilized on our surface and would be eliminated in the rinsing process and would thus not affect the single molecule force experiment adversely.

1.1.3. pH responsiveness of PEG_{114}-b-poly(L-lysine)$_{134}$-NH_2 determined by circular dichroism

The conformation of PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-NH_2 under different pH conditions was studied with circular dichroism spectrophotometry prior SPDP coupling assuming that addition of 2-pyridyl disulphide do not influence the secondary structure.

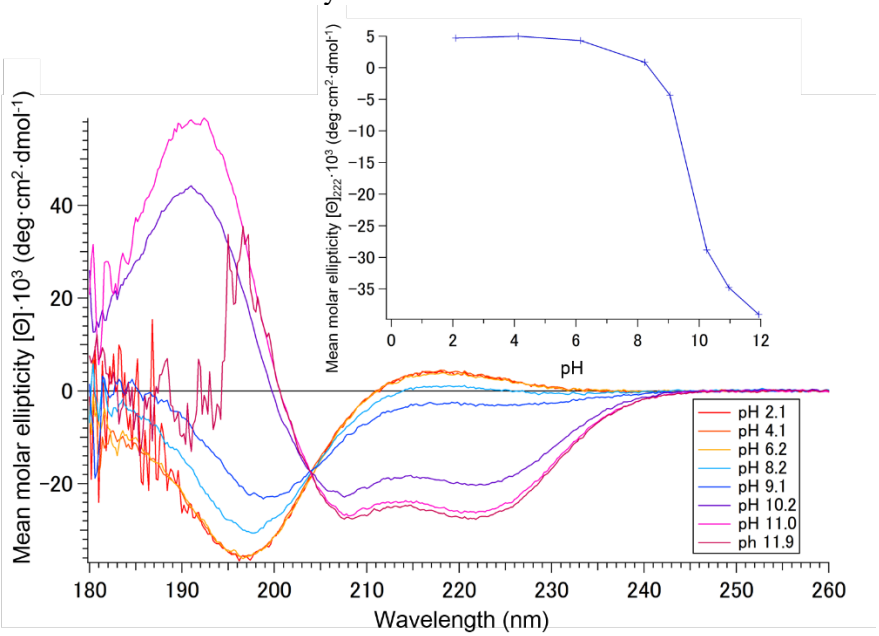

Figure S5. Circular dichroism spectra between 180 nm-260 nm of PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-NH_2 in different pH conditions from pH 2-12. Concentration=0.25 mg/ml; cell length=0.1cm; 20°C. The inset shows the variation of the mean molar ellipticity $|\theta_{mean\ molar}|$ at 222 nm with pH.

The obtained CD spectrum (Figure S5) shows that PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-NH_2 in aqueous solution is able to undergo a coil-helix transition in response to pH changes. At pH 9 and below, the copolymer adopts a random coil disorder state, indicated by a characteristic strong negative mean molar ellipticity $[\theta_{mean\ molar}]$ between 195-197 nm and a weakly positive $[\theta_{mean\ molar}]$at around 218 nm. From pH 10 and above, the molecule adopts a α-helix conformation, indicated by a characteristic positive $[\theta_{mean\ molar}]$ at 190 nm and two negative peaks at 208 nm and 222 nm respectively. A clear transition from a predominantly random coil to that of a α-helix conformation occurs rapidly between pH 9 and 10. This transition is almost completed at pH 11 and is completed at pH 12. The inset shows a graph following the evolution of the $[\theta_{mean\ molar}]$ at 222 nm with change in pH.

Using the expressions and values proposed by Luo and Baldwin,[3] the estimated helix contents were calculated at a value of 53.2% at pH 10, 67.5% at pH 11 and 70.9% at pH 12. It is to consider that such helix content is only a rough estimation because it is strictly incorrect to assume a fixed fractional helical percentage. The peptides in bulk are rather an ensemble of helices of varying, fluctuating lengths. In addition, it is important to keep in mind that the properties obtained for macromolecules in solution do not necessarily correspond to the behaviour of the macromolecule at the single molecule scale. Nonetheless, the CD spectra demonstrates that the molecule is able to undergo a conformational transition in response to pH and that most of the available polypeptide segment can fold into a helix.

1.2. Synthesis and characterization of PEG_{114}-*b*-poly(L-glutamic acid)$_{85}$-(2-pyridyl disulphide)

1.2.1. Synthesis of PEG-polypeptide Block Copolymers by Ring Opening Polymerization of Amino acid N-carboxyanhydrides.

PEG_{114}-*b*-poly(*L*-glutamic acid)$_{85}$-(2-pyridyl disulphide) was synthesized by the ring-opening polymerization (ROP) of γ-Benzyl-*L*-glutamate *N*-carboxyanhydride (Bn-Glu NCA) initiated by a PEG-NH_2 initiator (Figure S6). The resulting PEG_{114}-*b*-poly(γ-benzyl-*L*-glutamate) was then coupled with *N*-Succinimidyl-3(2-pyridyldithio) propionate (SPDP) and subsequently deprotected with H_2+ Pd/C yielding our final product (Figure S7).

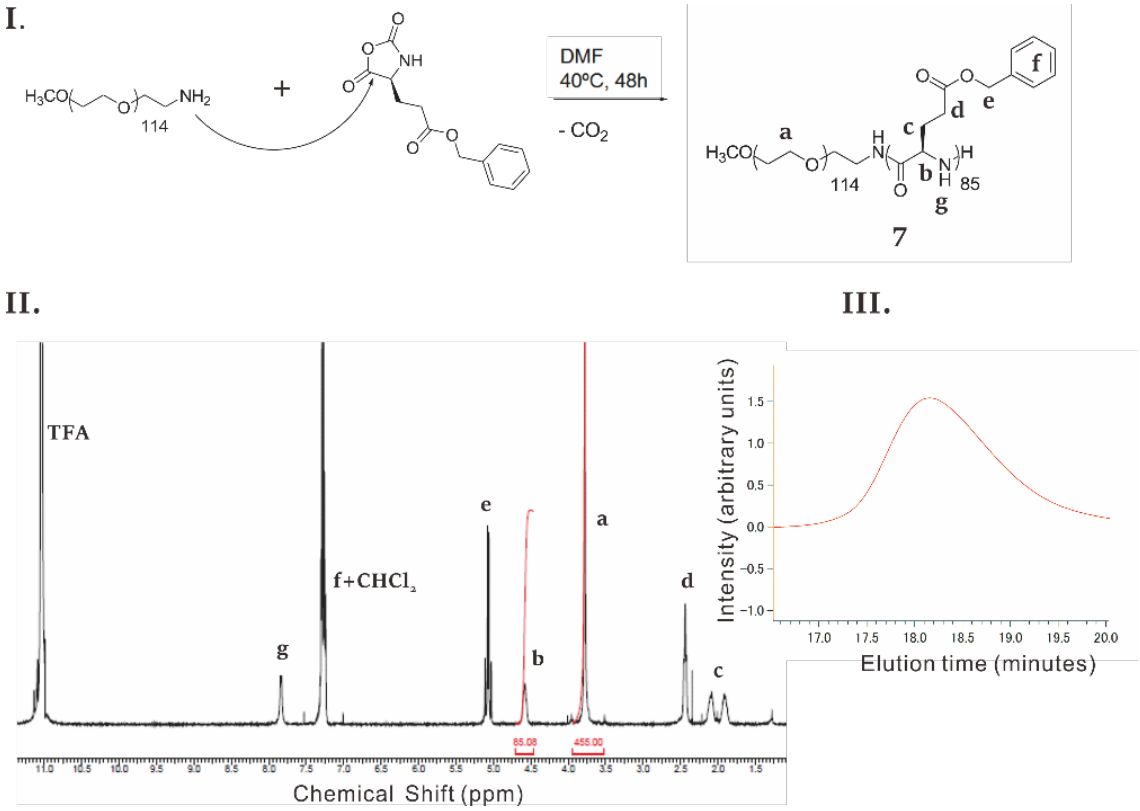

Figure S6. Initiation and growth of PEG-NH_2 initiated ROP of γ-Benzyl-*L*-glutamate *N*-carboxyanhydride. I.) ^{1}H NMR of PEG_{114}-*b*-poly(γ-benzyl-*L*-glutamate)$_{85}$-NH_2 in $CDCl_3$ + 15% TFA (Bruker 400 MHz). II.) SEC of PEG_{114}-*b*- poly(γ-benzyl-*L*-glutamate)$_{85}$-NH_2 in DMF containing 1g·L^{-1} LiBr , dispersity $Đ=\frac{Mw}{Mn}$=1.18; 60°C.

The resulting degree of polymerization (DP ≈ 85) was determined by comparing the integration of peak **b**, corresponding to the proton of the α-carbon of the peptide backbone (COC**H**RNH, 4.57 ppm) with that of the integration corresponding to the PEG_{114} (C**H$_2$**C**H$_2$**O, 3.78 ppm) labelled as **a** (Figure S6).

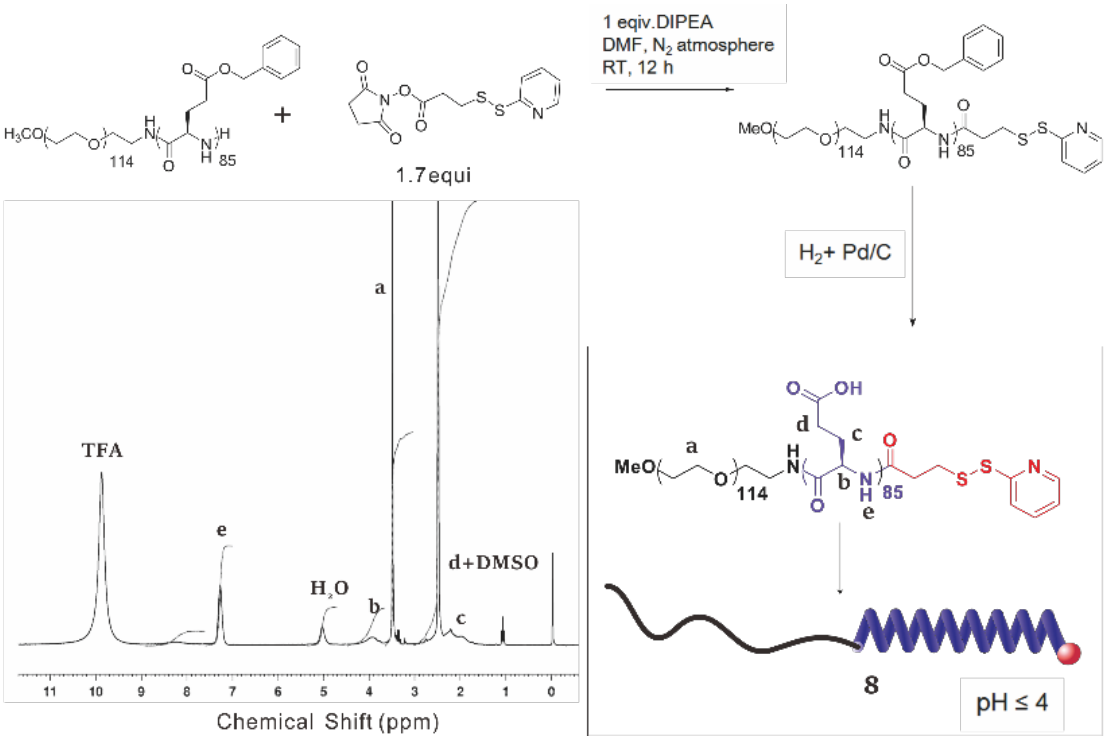

Figure S7. Deprotection of PEG_{114}-*b*-poly(γ-benzyl-*L*-glutamate)$_{85}$-(2-pyridyl disulphide). ^{1}H NMR of PEG_{114}-*b*-poly(*L*-glutamic acid)$_{85}$-(2-pyridyl disulphide) in DMSO-d_6 + TFA (Bruker 400 MHz).

1.2.2. pH responsiveness of PEG_{114}-poly(L-glutamic acid)$_{85}$-(2-pyridyl disulphide) determined by circular dichroism

The CD spectrum (Figure S8) monitored in aqueous solutions shows that the copolymer is able to undergo a coil-helix transition in response to pH changes. At pH 6.3, the molecule adopts a random coil structure, indicated by a characteristic strong negative mean molar ellipticity [$\theta_{mean\ molar}$] between 195-197 nm and a weakly positive [$\theta_{mean\ molar}$] at around 218 nm. At pH 2.3, the block copolymer adopts a α-helix conformation, indicated by a characteristic positive [$\theta_{mean\ molar}$] at 190 nm and two negative peaks at 208 nm and 222 nm respectively. The pH of transition should be at pH ≈ 4.5 as previously determined.[4]

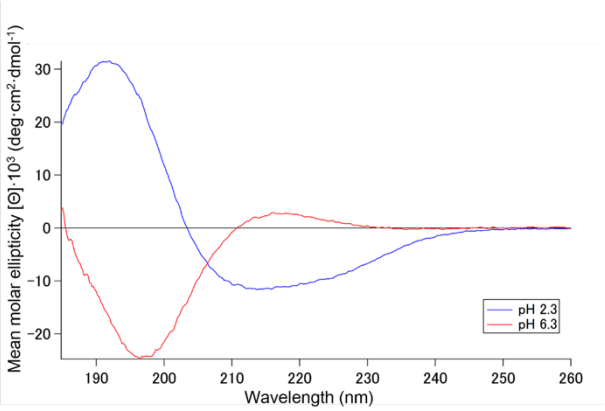

Figure S8. Circular dichroism spectrum between 180 nm-260 nm of PEG_{114}-*b*-poly(*L*-glutamic acid)$_{134}$-(2-pyridyl disulphide) in pH 2.3 and 6.3 Concentration=0.1 mg/ml; cell length=0.1 cm; 25°C.

Using the expressions and values proposed by Luo and Baldwin,[3] the estimated helix content was calculated at a value of 28% at pH 2.3. The percent helicity was dramatically lower in comparison with the PEG_{114}-poly(*L*-Lysine) system (≈ 70%). A study done by Harada, Cammas and Kataoka[5] on a PEG_{98}-poly(*L*-Lysine)$_{19}$ molecule offered an explanation of a helix stabilizing effect observed for a poly(*L*-lysine) copolymerized with PEG. The stabilization effect is due to the increasing hydrophobicity of the lysine side chain with deprotection that causes the PEG segment to form a protective shell around the lysine, facilitating its segregation from the aqueous medium. The association with PEG was shown to protect the lysine helix in the presence of urea, by isolating and maintaining the helical structure. Despite copolymerization with PEG, there is a limited stabilization effect for the PEG-*b*-poly(*L*-glutamic acid) as the side chains are negatively charged above their pKa values. The negatively charged side chains would rather repel the PEG, which has long-pair electrons on the oxygen atoms along the chain. In comparison, the lysine side chains are positively charged at lower pH and forms an attractive interaction with the lone-pair electrons on the oxygen atoms along the PEG chain. In addition due to a shorter aliphatic chain and a carboxyl moiety, the poly(*L*-glutamic acid) is highly hydrophilic. The polypeptide is also coupled to long PEG, which in the absence of the stabilization effect is thought to further solubilize the diblock and disrupt the helix formation.

The bulk solution properties do not necessarily correspond to the properties of the molecule at the single molecule scale. In addition, the helicity of an unprotected molecule is always much lower as a result of frayed ends in solution. The two extremities of the helix are well dissolved in the solution, decreasing the overall helical content.[6] In the SMFS experiment the end of the molecule will be grafted on a gold surface, and the other extremity pulled by an AFM tip, thus the helical content is expected be much higher for each molecule.

1.3. Conditions needed to induce a α-β transition of PEG_{114}-*b*-poly(L-lysine)$_{134}$-NH_2 and investigation of its reversibility determined by circular dichroism

As described in section 1.1.3., the pH of coil-helix transition for PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-NH_2 was determined to occur rapidly between pH 9 and 10 and the estimated helix content was 70.9 % at pH 12, calculated using the expression proposed by Luo and Baldwin.[3] The β-form of poly(*L*-lysine) has been shown to be most stable at 50°C.[7-9] Four different experiments were thus conducted to study the behaviour of PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-NH_2 undergoing a α–β transition and the reversibility of

such a transition.

In the first study, the temperature was varied from 20°C to 60°C when the solution pH was kept constant at pH 12 to determine the minimum temperature conditions needed to induce the α–β conformational transition. From the CD spectra obtained (Figure S9a) it is evident that at pH 12, temperature of at least 50°C is needed to induce the transition. The addition of the PEG segment does not seem to have an effect on the pH or temperature of transition as the temperature of transition is the same as reported in literature.[9] The inset (Figure S9b) shows the results obtained when the helix-coil transition behaviour of PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-NH_2 was observed at 20°C when the pH conditions were varied.

In the second study, the pH was varied incrementally from 2 to 12, while the temperature was kept at 50°C. This was done to determine the exact temperature and pH conditions needed to induce the α–β transition.

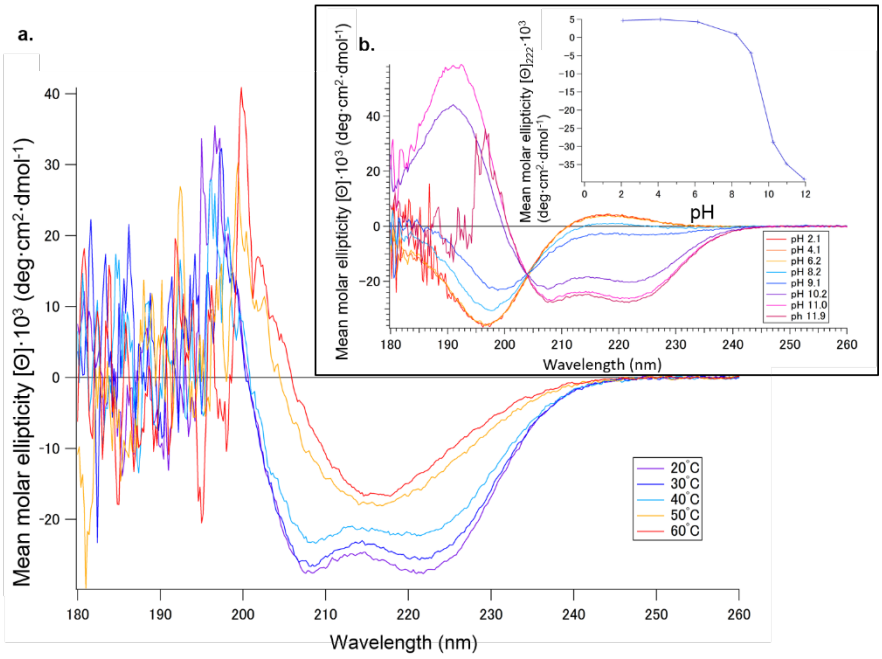

Figure S9. Circular dichroism spectra between 180 nm-260 nm of PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-NH_2 at a constant pH 12 for temperatures between 20°C to 60°C. The inset shows the circular dichroism spectra obtained when the pH was varied incrementally from pH 2-12 when the temperature was kept constant at 20°C. Concentration = 0.25 mg/ml; cell length = 0.1cm.

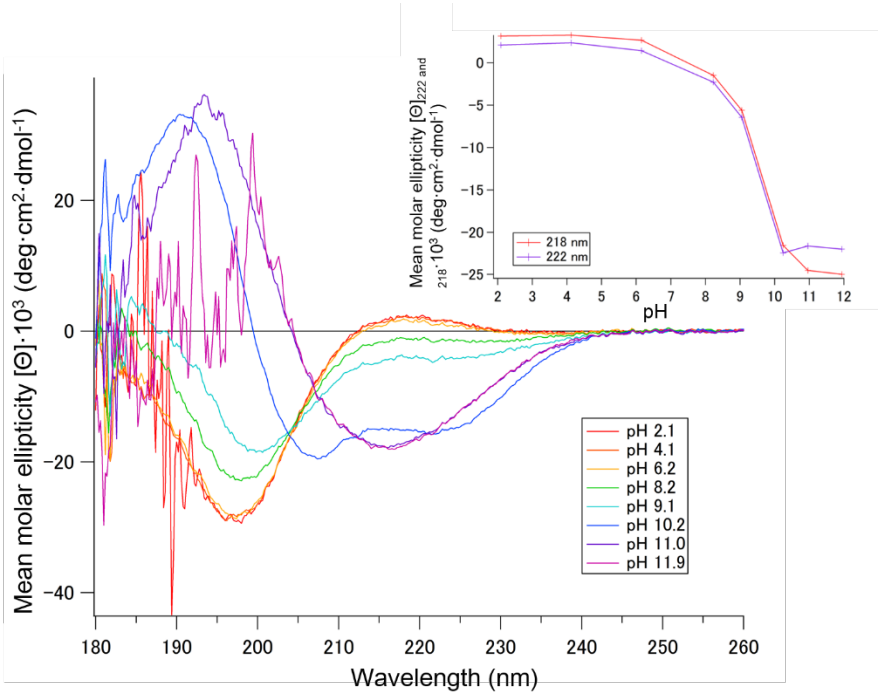

Figure S10. Circular dichroism spectra between 180 nm-260 nm of PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-NH_2 in different pH conditions from pH 2-12 at 50°C. Concentration = 0.25 mg/ml ; cell length = 0.1cm ; 50°C. The inset compares the variation of the mean molar ellipticity $|\theta_{mean\ molar}|$ at 222 nm with the mean molar ellipticity $|\theta_{mean\ molar}|$ at 218 nm for different pH conditions.

The CD spectra obtained at 50°C and at differing pH conditions (Figure S10) demonstrate that the α–β transition can only occur when both conditions, basic pH and elevated temperatures, are fulfilled. The lysine side chains must be fully deprotonated before transition can occur. At pH 9 and below, the molecule adopts a random coil conformation, indicated by a strong negative peak between 195-197 nm and a weakly positive peak at 215 nm. At pH 10, the typical characteristic trace indicative of a α-helix can be observed, suggesting that at this pH the molecule is caught at the threshold of undergoing the transition. From pH 11 onwards the appearance of the characteristic negative mean molar ellipticity at 218 nm can be observed, indicative of the anti-parallel β-sheet. The inset of Figure S10 is a graph comparing the variation of the mean molar ellipticity at 222 nm corresponding to the α-helix with that of the mean molar ellipticity at 218 nm corresponding to the β-sheet for different pH conditions. A clear shift can be perceived from pH 11 where the peak at 222 nm stagnates in value, while the signal corresponding to 218 nm becomes increasingly negative, signifying the formation of the β-sheet conformation.

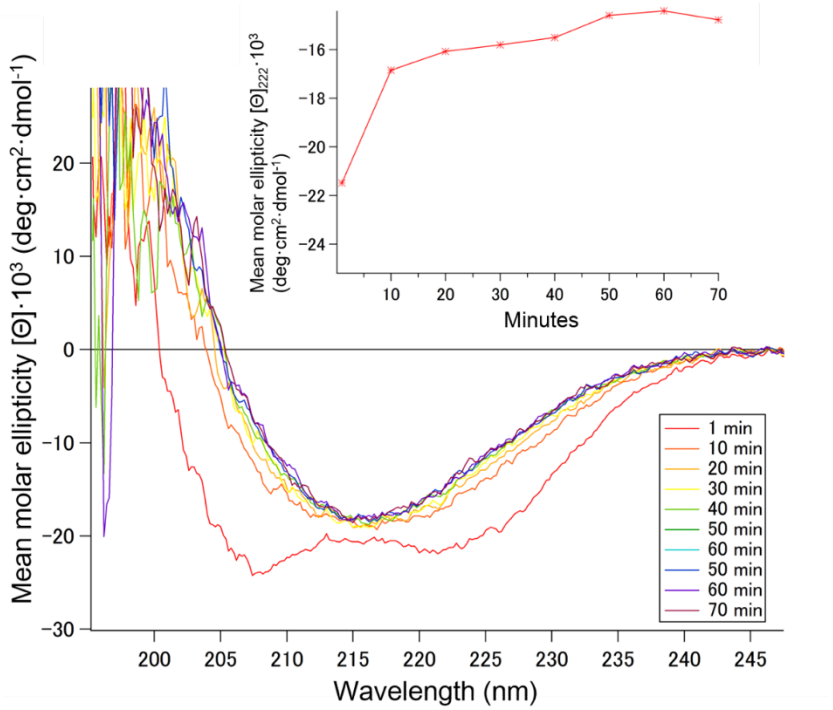

Figure S11. Circular dichroism spectra between 180 nm-260 nm of PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-NH_2 at pH ≈ 12 at 50°C from 1 min-70 min. Concentration = 0.25 mg/ml ; cell length = 0.1 cm ; pH ≈ 12 ; 50°C. The inset shows the evolution of the mean molar ellipticity $|\theta_{mean\ molar}|$ at 222 nm with time.

In the third study the evolution of the transition was followed at 50°C and measurements were taken every 10 minutes to verify the amount of time needed for the transition to occur. In all the measurements, the value of the negative mean molar ellipticity at 218 nm was followed, which indicated the formation of the anti-parallel β–sheet structure.[7] The peak at 218 nm formed at the expense of the α-helical structure indicated by the disappearance of the two negative peaks at 208 and 222 nm.

The spectra (Figure S11) demonstrates that at 50°C the majority of the α–β transition happens within a time span of 10 minutes. Within 10 minutes of starting the measurement, the characteristic double minima at 208 nm and 222 nm indicative of the α-helical conformation is lost. After this time, the transition is still ongoing but to a lesser extent. The inset displays the variation of the peak at 222 nm verifying the loss of the helical conformation with time.

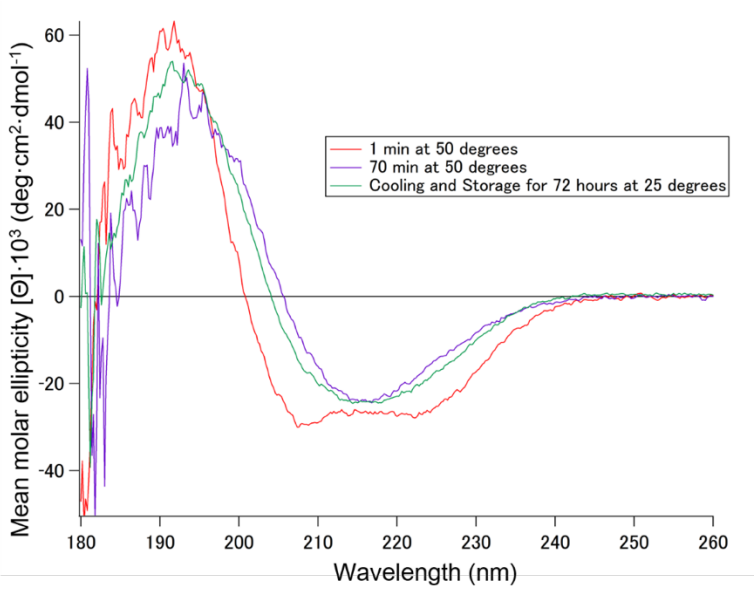

Figure S12. Circular dichroism spectra between 180 nm-260 nm of PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-NH_2 at pH ≈ 12. Three different spectra are compared where measurements were taken after 1 minute at 50°C pH 12 (red line), after 70 min at 50°C pH 12 (purple line) and after cooling and storage for 72 hours at 25°C. Concentration = 0.25 mg/ml; cell length = 0.1cm ; pH ≈ 12 ; 25°C and 50°C.

Finally, in the fourth study (Figure S12) the reversibility of the α–β transition was investigated by comparing three different measurements taken immediately prior to the α–β transition (1 minute at 50°C), after the transition into the anti-parallel β-sheet structure had occurred (70 min at 50°C), and after cooling and storage for 72 hours at 25°C to determine whether the antiparallel β-sheet structure could be retained.

After 1 minute at 50°C, the α-helical structure of the poly(*L*-lysine) segment is still evident in the double minima at 208 nm and 222 nm and the positive ellipticity at 190 nm. After 70 minutes at 50°C the formation of the anti-parallel β-sheet is indicated by the formation of a single minima at 218 nm and the shift of the positive ellipticity to 196 nm. The spectrum of the sample after cooling and storage indicates that although some key characteristics of the anti-parallel β-sheet structure are retained, indicated by the preservation of the negative minima at 218 nm, the shift of the positive peak back to 190 nm, which is indicative of a α-helical structure, suggests that the structure is an intermediate between both states. The results suggest that within the time frame of the measurements the α–β transition is not reversible, but neither could the β-sheet structure be retained. Instead the resulting structure after cooling and storage is an intermediate structure, which have characteristics pertaining to both α-helical and β-sheet structures. This is in line with also what was reported in literature where the once folded β-sheet structure was shown to not easily revert back to the α-helical conformation after cooling.[8, 9]

1.4. Materials and methods for the polymerization and characterization of PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-(2-pyridyl disulphide) and PEG_{114}-*b*-poly(*L*-glutamic acid)$_{85}$-(2-pyridyl disulphide)

The chemicals and reagents used for the synthesis of PEG-NH_2 and polymerization and coupling reactions to obtain PEG_{114}-*b*-poly(*L*-lysine)$_{134}$-(2-pyridyl disulphide) are listed below. Unless otherwise stated, the chemicals were used as received.

Chemicals	Manufactuer	Prior processing
1,4 dioxane	Sigma Aldrich	
4-Dimethylaminopyridine (DMAP)	Sigma Aldrich	
CaH_2	Sigma Aldrich	
Dichloromethane (anhydrous)	Sigma Adrich	Kept in solvent purification system
Diethylether	Sigma Aldrich	
ε-TFA-*L* -Lysine NCA	IsoChem	Stored in a glove box and used as supplied
γ-benzyl-*L* -glutamate *N* -carboxyanhydride	IsoChem	Stored in a glove box and used as supplied
HCl 37%	Sigma Aldrich	
KOH 85%	Merck	
Magnesium Sulphate (anhydrous)	Alfa Aesar	
Methanesulfonyl chloride (MsCl)	Sigma Aldrich	
Methoxy poly(ethylene glycol) , average M_n= 5000 g mol^{-1}	Fluka	Dissolved in anhydrous toluene and the moisture was azeotropically distilled
NaOH 97%	Alfa Aesar	
NH_4OH 28%	Prolabo	
N,N-Diisopropylethylamine (DIPEA)	Alfa Aesar	
N,N -Dimethylformamide (DMF 99.8% anhydrous)	Sigma Adrich	Dried over CaH2 (Sigma Aldrich) and cryodistilled.
N,N,N -Triethyl amine	Fischer	Dried over CaH_2 (Sigma Aldrich) and cryodistilled
N -Succinimidyl 3-(2-pyridyldithio)-propionate (SPDP)	Thermo Fischer	
Tetrahydrofuran (THF)	Sigma Aldrich	
Toluene (anhydrous)	Sigma Aldrich	Kept in solvent purification system

1.4.1. Synthesis of PEG_{114}-SO_3CH_3 from PEG-OH (Mn= 5000 g mol^{-1})

10.0 g (2.0 mmol) of dry PEG-OH was dissolved in 15 ml of anhydrous dichloromethane under argon atmosphere. 840 μL (5.99 mmol) of dry triethylamine was added dropwise to the solution followed by 48.9 mg (0.40 mmol) of DMAP. After all the reactants were completely solubilized the solution was cooled to 0ºC over an ice bath. Finally, 387 μL (5.0 mmol) of MsCl was added dropwise to the cooled solution and the reaction was left for 18 h on ice. The resulting solution was washed twice with 4 ml of 2 M HCl. The phase containing the PEG-mesylate intermediate was then concentrated to approximately one fourth of its initial volume and precipitated in 10 times the volume of ice-cold diethyl ether. The resulting cream-white solid was then recovered by filtration and dried under vacuum overnight to remove traces of solvent before proceeding to the next step yield = 91.2 % (9.12 g). The chemical structure of the intermediate was confirmed by ^{1}H NMR (Figure S1), and the full functionalization was confirmed by comparing the integration of the protons of the methyl group labelled as **a** (C**H$_3$**O, 3.38 ppm) with that of the ethyl protons beside the mesylate **c** (C**H$_2$**OS(O_2)CH_3, 4.38 ppm).

1.4.2. Synthesis of PEG_{114}-NH_2 (Mn = 5000 g mol^{-1})

5.00 g (1 mmol) of PEG-SO_3CH_3 was added to 100 ml of 28 % aqueous ammonia solution and the reaction was left to stir at 25ºC in a tightly sealed round-bottom flask for 5 days. Subsequently, the lid was left ajar and the ammonia was left to evaporate in air over the course of 2 days. The pH of the solution was adjusted to 13 by the dropwise addition of freshly prepared 5 M NaOH, and the polymer was extracted three times in 40 ml of dichloromethane. The combined dichloromethane phase was then washed with 10 ml of brine and dried with the addition of dry magnesium sulphate. Thereafter, the dichloromethane phase containing the polymer was concentrated to less than one fourth of its volume, and precipitated in ice-cold diethylether. The product was dried under vacuum, and recovered as a pure-white powder, yield = 72 % (3.6 g). The full functionalization was confirmed by ^{1}H NMR by comparing the integration of the protons on the methyl end-group labelled as **a** (C**H**$_3$O, 3.38 ppm) with that of the ethyl protons beside the amine end-function **c** (OC**H**$_2$$NH_2$, 3.19) (Figure S1).

1.4.3. Synthesis of PEG_{114}-b-poly(N^{ε}-trifluoroacetyl-L-lysine)$_{134}$-NH_2 by ring-opening polymerization of N-trifluoroacetyl-L-lysine N-carboxyanhydride

The synthesis of the copolymer was performed by NCA polymerization under low temperature conditions. Immediately prior to the reaction, 0.2 g (0.04 mmol) of PEG-NH_2 (Mn = 5000 g mol^{-1}) from the previous synthesis was dissolved in 1,4 dioxane and any remaining moisture was azeotropically distilled out. After the PEG-NH_2 was completely dry it was redissolved in 2 ml of freshly distilled DMF. 1.18 g (4.4 mmol) of N^{ε}-TFA-*L*-Lys-NCA was recovered in a flame-dried schlenk under inert atmosphere, and dissolved in 13 ml of freshly distilled DMF. Immediately after all of the monomer had dissolved in DMF (10 min<), the monomer solution was added to the schlenk containing the PEG-NH_2 initiator. The mixture was left under dynamic vacuum for 10 minutes to allow the generated CO_2 to escape. Subsequently, the schlenk was sealed off and the polymerization was left to run for 5 days at 5ºC under constant stirring and monitored by ^{1}H NMR. After 5 days, half of the volume of the DMF was extracted through cryo-distillation, after which the polymer was precipitated in 10 times the volume excess of ice-cold diethyl ether. The precipitated PEG_{114}-*b*-poly(N^{ε}-trifluoroacetyl-*L*-lysine)$_{134}$-NH_2 was recovered by centrifugation and finally dried under vacuum.

The resulting degree of polymerization (DP ≈ 134) was determined by comparing the integration of the peak corresponding to the CH_2 signal nearest to the trifluoroacetyl group labelled as **d** (C**H**$_2$NHCOCF_3, 3.13 ppm) with that of the integration corresponding to the PEG_{114} (C**H**$_2$C**H**$_2$O, 3.51 ppm) labelled as **a** (Figure S3). Molecular mass dispersity was obtained by SEC in DMF (LiBr, 80°C): Đ = 1.18.

1.4.4. Coupling of PEG_{114}-b-poly(N^{ε}-trifluoroacetyl-L-lysine)$_{134}$-NH_2 with N-Succinimidyl 3-(2-pyridyldithio)-propionate (SPDP)

0.05 g (0.0014 mmol) of PEG_{114}-*b*-poly(N^{ε}-trifluoroacetyl-*L*-lysine)$_{134}$-NH_2 (was dissolved in 400 µl of DMF. In a separate vial, 0.89 mg (0.0029 mmol) of *N*-Succinimidyl 3-(2-pyridyldithio)-propionate (SPDP) was dissolved in 100 µl of DMF. To the vial containing the copolymer 0.49 ml (0.0029 mmol) of DIPEA (Sigma Aldrich) was added and was left to stir for no more than 15 minutes. Finally, the SPDP was carefully added to the vial containing the copolymer and the DIPEA. The reaction was left to stir for 3 days, after which reaction was diluted with water and dialyzed with 3500 Dalton membranes Spectra/Por® for a minimum of 4 days. Subsequently the samples were collected and lyophilized.

1.4.5. Deprotection of PEG_{114}-b- poly (N^{ε}-trifluoroacetyl-L-lysine) $_{134}$-(2-dipyridyl disulphide)

0.025 g (0.00071 mmol) of PEG_{114}-*b*-poly(N^{ε}-trifluoroacetyl-*L*-lysine)$_{134}$-(2-dipyridyl disulphide) was dissolved in 1.08 ml of ethanol containing 120 µl of 5 M KOH. The turbid mixture as left to stir overnight in a water bath set to 35ºC. The mixture was dialyzed with 3500 Dalton membranes from Spectra/Por®. Subsequently the samples were collected and lyophilized.

1.4.6. Synthesis of PEG_{114}-b- poly(γ-benzyl-L-glutamate)$_{85}$-NH_2 by ring-opening polymerization of γ-Benzyl-L-glutamate N-carboxyanhydride

Briefly, α-methoxy-ω-amino poly(ethylene glycol) (CH_3O-PEG-NH_2, 5000 g/mol, RAPP Polymere, Germany; 0.5 g, 0.1 mM) was dissolved in 2 mL dioxane, freeze-dried and dissolved in dry DMF (0.1 g/mL). In a glovebox, γ-benzyl-*L*-glutamate *N*-carboxyanhydride (2.6 g, 10 mM) was introduced into a flame-dried Schlenk flask and dissolved in anhydrous DMF (0.1 g/mL). This solution was added to the PEG solution under vacuum and the mixture was stirred for 48 hours at 40°C in an oil bath. The polymerization medium was concentrated by cryodistillation and the copolymer was recovered by precipitation into cold diethyl ether. The polymer was recovered as a white powder after three washings with diethyl ether and drying under dynamic vacuum for 24 hours. The resulting degree of polymerization (DP ≈ 85) was determined by comparing the integration of peak **b**, corresponding to the proton of the α-carbon the peptide backbone (COC**H**RNH, 4.57 ppm) with that of the integration corresponding to the PEG_{114} ($C\mathbf{H_2}C\mathbf{H_2}O$, 3.78 ppm) labelled as **a** (Figure S7). Molar mass dispersity was obtained by SEC in DMF (LiBr, 60°C): Đ = 1.18.

1.4.7. Coupling of PEG_{114}-b-poly(γ-benzyl-L-glutamate)$_{85}$-NH_2 with N-Succinimidyl 3-(2-pyridyldithio)-propionate (SPDP)

PEG-*b*-PBLG was modified in bulk using succinimidyl 3-(2-pyridyldithiolpropionate) (SPDP, Pierce). Briefly, PEG-*b*-PBLG copolymer (1 g) was dissolved in anhydrous DMF (0.1 g/mL) and *N*,*N*-diisopropylethylamine (DIPEA) (6 µL ; 1 eq. per amine function) was added. After 5 min stirring, SPDP (20 mg; 1.7 eq.) was added and the mixture was stirred overnight at room temperature under N_2 atmosphere. The resulting product was recovered by precipitation in cold diethyl ether and dried under vacuum.

1.4.8. Molecular Characterization by ^1H NMR

All ^{1}H NMR experiments were performed on a Bruker Avance 400 MHz spectrometer using TMS as a zero-point standard. The machine was equipped with a Bruker multinuclear z-gradient direct probe head producing gradients in the z-direction with a strength of 53.5 $G \cdot cm^{-1}$. The spectra were acquired with a DI of 9 seconds and 32 scans. ^{1}H NMR samples were dissolved at 5 $mg \cdot ml^{-1}$ in $CDCl_3$, $(CD_3)_2SO$ and D_2O.

1.4.9. Size-Exclusion Chromatography.

Size-exclusion chromatographs were performed using DMF LiBr 1$g \cdot L^{-1}$ at 80 °C as the eluent, at a flow rate of 0.80 $ml \cdot min^{-1}$. A SHODEX KD-804 column was used and calibrated with PEG standards. Dilute samples of 5.0 $mg \cdot ml^{-1}$ were prepared and injected.

1.4.10. Circular Dichroism Spectroscopy

The CD experiments were performed using a JASCO J-815 spectrometer equipped with a JASCO CDF-426S Peltier temperature control system. The scans were recorded between wavelengths of 180 to 260 nm and are a result of three accumulations. Block copolymer solutions were prepared at a concentration of 0.25 mg·ml^{-1} and 0.1 cm quartz cuvettes were used. The pH 2-12 was adjusted by the addition NaOH or HCl (0.1 M and 1 M). The base or acid was added by using a micropipette and the exact amount of added liquid was recorded so that the difference in dilution could be taken into consideration. All solutions were filtered with a 0.22 µm syringe filter before use.

1.5. Theoretical size of the polypeptides

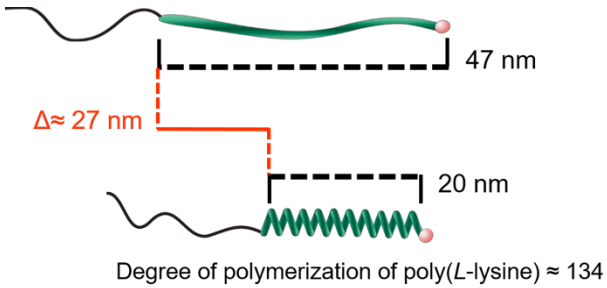

Figure S13. Theoretical difference in length between the folded and unfolded poly(*L*-Lysine) α-helix segment within the synthesized PEG$_{114}$-*b*-poly(*L*-lysine)$_{134}$-(2-pyridyl disulphide) block copolymer calculated from the degree of polymerization obtained by ^{1}H NMR.

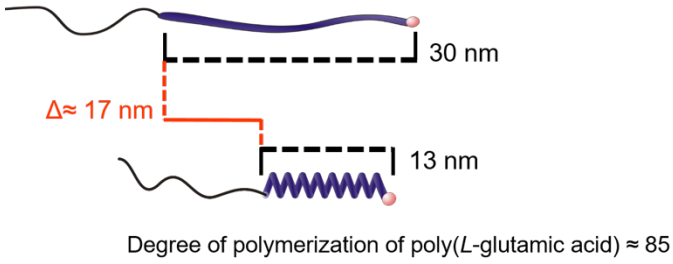

Figure S14. Theoretical difference in length between the folded and unfolded poly(*L*-glutamic acid) α-helix segment within the synthesized PEG$_{114}$-*b*-poly(*L*-glutamic acid)$_{85}$-(2-pyridyl disulphide) block copolymer calculated from the degree of polymerization obtained by ^{1}H NMR.

1.6. Single-molecule force spectroscopy experiments for PEG$_{114}$-*b*-poly(*L*-lysine)$_{134}$-(2-pyridyl disulphide) in the β-form

Previous works on PLys mentioned below predict that the grafted molecule will be able to successfully fold into a β-sheet structure. As discussed in the main text, as the length of the lysine segment is very long, it is folded into a higher order structure.[10,11] Longer helices with more turns between the helical segments have been indicated to promote greater β-sheet propensity.[12] In fact, the length of the starting α-helix is so paramount that if the lysine segment was too short, the molecule would not able to undergo a conformational transition despite elevated temperatures.[11] This effect has

been attributed to the helical segments interacting favourably in an antiparallel manner resulting in water molecules to be ejected out from the inter-helical space, resulting in a more hydrophobic environment.[11] This increased hydrophobicity promotes the formation of the β structure, therefore if the lysine segment is not long enough for helix-helix adhesion to take place the α–β transition does not occur. Single molecules of poly(*L*-lysine) is predicted to also exhibit the β-structure as β-poly(*L*-lysine) has also been indicated to form by intramolecular interactions[7,8] and not just by intermolecular interactions. The once folded β-poly(*L*-lysine) has been also shown to not easily revert back to the α-helical conformation after transition. In optical rotary dispersion and CD studies[8,9], it was found that when β–poly(*L*-lysine) is cooled from 50 to 25°C, the poly(*L*-lysine) did not to regain its helical conformation.

2. Supplementary Figures

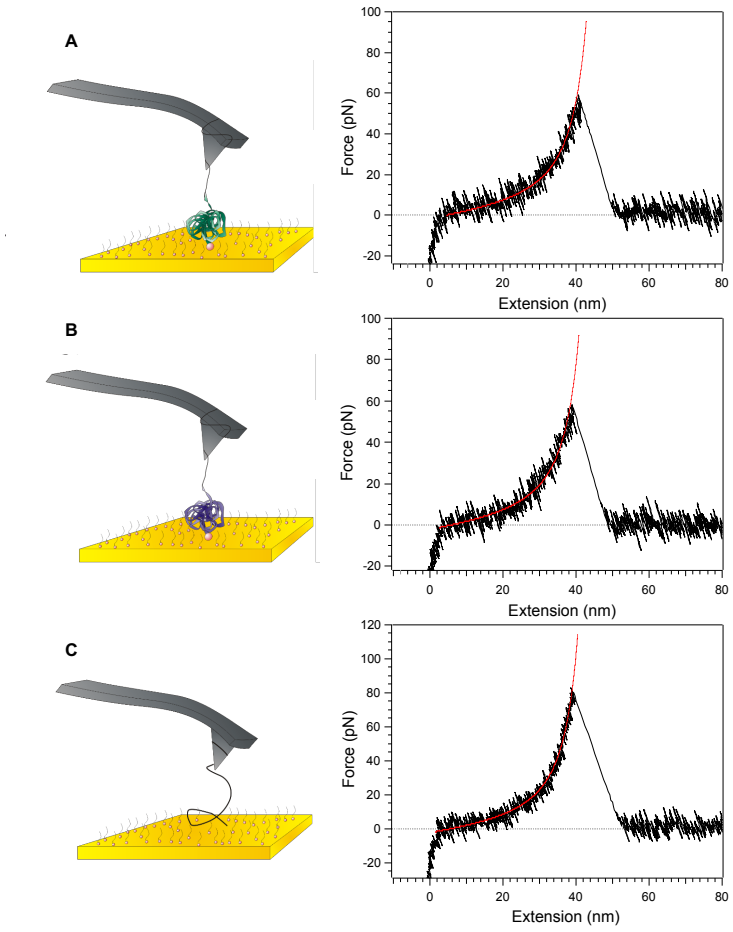

Figure S15. Force-extension profiles and schematic representations for poly(*L*-lysine), poly(*L*-glutamic acid), and PEG alone as a control, when each of them is in a random coil conformation. A. poly(L-lysine) at pH 7. B. poly(L-glutamic acid) at pH 7. C. PEG at pH 7. The red line indicates a fit using the WLC model, which predicts the behavior of an ideal random coil polymer.

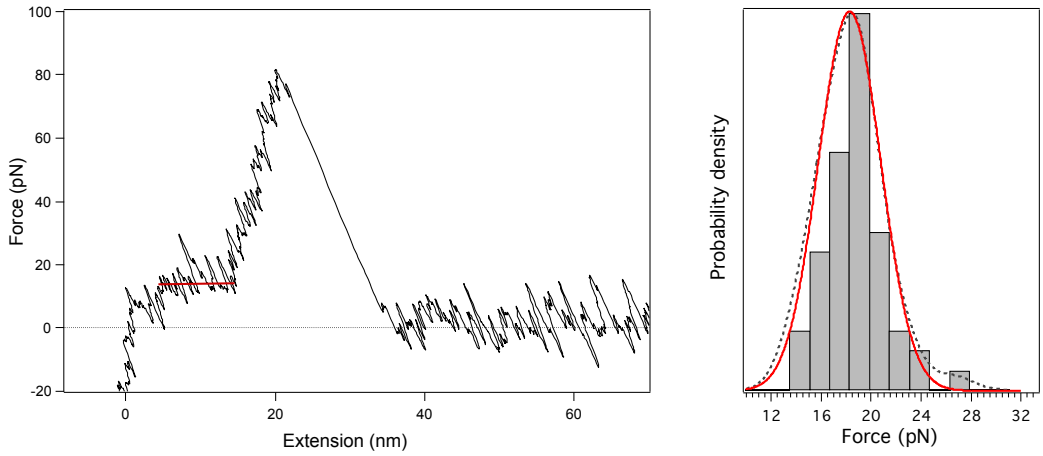

Figure S16. Force-extension curve for poly(*L*-glutamic acid) in a α-helix conformation, showing a plateau (20% of the cases), and the corresponding histogram of plateau force. The probability density function and Gaussian distribution give a plateau force of 19 ± 2 pN (± s.d, n=55).

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