Electronic Supplementary Information

Poly(N-vinylpyrrolidone-b-( $\gamma$ -benzyl-L-glutamate)) – Synthesis and self-assembly into pH-sensitive micelles.

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#### Experimental procedures

#### Characterization

<sup>1</sup>**H NMR** spectra were acquired in  $CDCI_3$  (unless specified otherwise) with a Varian VXR-Unity (300 MHz) spectrometer. All chemical shifts are reported in parts per million (ppm) with tetramethylsilane (TMS) as an internal reference.

**Size exclusion chromatography (SEC)** analysis was carried out on a dimethylacetamide (DMAc) solvent system using a flow rate of 1.0 mL/min. The instrument setup consisted of a Shimadzu LC-10AD pump, a Waters 717plus autosampler, a column system fitted with a 50x8 mm guard column in series with three 300x8 mm, 10 µm particle size GRAM columns (2 x 3000Å and 100Å) obtained from PSS, a Waters 2487 dual wavelength UV detector and a Waters 410 differential refractive index (DRI) detector all in series. 100 µL injection volumes are sampled individually with the oven temperature of the column and DRI detector kept at 40 °C. The solvent was stabilized with 0.05% BHT (*w/v*) and 0.03% LiCl (*w/v*), and samples were filtered through a 0.45 µm GHP filter to prevent any impurities from entering the system. Calibration was done using PMMA standards (Polymer Laboratories) ranging from 690 to 1.2 x 10<sup>6</sup> g/mol. Data acquisition was done using Millennium<sup>32</sup> software, version 4.

**Fluorescence spectroscopy** was measured on a Perkin-Elmer LS-50B luminescence spectrometer. FL Winlab V4 was used for data acquisition and processing.

The average hydrodynamic diameter of the micelles was determined *via* **dynamic light scattering (DLS)**. This was achieved with a Zetasizer nano ZS90 instrument. Particle sizes were detected at a 90° scattering angle with a 4 mW He-Ne laser and a 633 nm incident beam (Malvern Instruments, UK). The final particle size and particle size distribution were obtained from three measurements, each comprising 10–15 sub-runs. The particle size and particle size distribution were calculated using CONTIN analysis. Results are presented as the Z-average particle diameter.

The **transmission electron microscopy (TEM)** images were recorded with an FEI Tecnai TF20 field emission gun transmission electron microscope (FEG-TEM) operated at 200 keV. Prior to imaging, the samples were stained with

uranyl acetate to induce contrast in the sample during imaging. The uranyl acetate stain consisted of 2 wt% uranyl acetate dissolved in deionised water. During staining, one drop of sample was placed on a carbon-coated copper grid and left for 60 seconds. The carbon-coated copper grids were glow-discharged beforehand to induce hydrophilicity in the carbon layer of the copper grid, which assists in the dispersion of the sample prior to staining. After 60 seconds the excess sample was blotted using filter paper and the stain applied by dipping the sample containing copper grid in the stain solution. The excess stain was then blotted and the sample placed under xenon light to dry.

# Materials

Potassium O-ethyl xanthate (95 %, Merck), tryptamine (> 99%, Sigma-Aldrich), 2-bromopropionyl bromide (97%, Fluka), sodium hydroxide (Saarchem) and anhydrous magnesium sulphate (Merck) were used as received. Tetrahydrofuran (THF) (Seccosolv<sup>TM</sup>), diethyl ether (Seccosolv<sup>TM</sup>), *n*-pentane (for synthesis) and triethylamine (for synthesis) were all purchased from Merck and used as received. Dichloromethane (DCM, KIMIX, CP-grade, 99.5 %.) and ethanol (KIMIX, CP-grade, 99.5 %) were dried over molecular sieves (4 Å, 1.6 mm pellet). L-glutamic acid (ReagentPlus), benzyl alcohol (anhydrous, 99.8%), bis(trichloromethyl) carbonate (triphosgene) (99%), phosphorous pentoxide (with MOI) and Nile Red (bioreagent, > 98%), ethyl acetate (anhydrous, 99.8%) and hydrochloric acid (HCl, 37 %) were purchased from Sigma-Aldrich and used without further purification. The ethyl acetate was used directly from the bottle and stored under an inert, dry atmosphere.

Pyridine (99%, Across Organics) and hydrobromic acid (48%, Fluka) were used as received. Phosphate buffered saline tablets (Merck) were used to make a PBS

solution.

# **RAFT** agent synthesis

The RAFT agent is an O-ethyl xanthate with a tryptamine-based R-group. This introduces a fluorescent end-group in the PVP homopolymer, with a maximum excitation wavelength at 283 nm and maximum emission wavelength at 350 nm.



Scheme S1 Synthesis of fluorescent tagged RAFT CTA, X21 (1)

#### Synthesis of *N*-(2-(1*H*-indol-3-yl)ethyl)-2-bromopropanamide (4)

Tryptamine (2.013 g, 12.56 mmol) and triethylamine (1.904 g, 18.81 mmol) were dissolved in dichloromethane (20 mL) in a round bottom flask. The flask was cooled with an ice bath while 2-bromopropionyl bromide (2.413 g, 11.18 mmol) was dissolved in dichloromethane (DCM) (10 mL) and added dropwise *via* a dropping funnel over approximately 10 min to the tryptamine solution. After the complete addition of the bromide solution, the ice bath was removed and the solution left stirring at room temperature for 16 hours.

A dark brown solution was obtained with a white precipitate ( $Et_3N^+HBr^-$ ), which was filtered off. Diethyl ether (200 mL) was added to the filtrate, which resulted in a heterogeneous solution, this cleared to an orange solution after an initial wash with 50 mL 1M HCl. The solution was then washed with a further 50 mL 1M HCl, 1M aqueous NaOH (50 mL) and finally with distilled water (50 mL) after which the organic phase was dried over anhydrous magnesium sulphate. The solvent was removed *via* rotary evaporation under high vacuum, resulting in a crystalline orange solid, which was subsequently redissolved in ethyl acetate and recrystallized from DCM. The product was left at 4 °C to ensure complete recrystallization. Yield: 2.53 g, 8.57 mmol, 68%.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, δ, ppm ): 8.26 (s, 1H, -CHN**H**), 7.60 (m, 1H, ArH ), 7.37 (m, 1H, ArH), 7.26 – 7.08 (m, 2H, ArH), 7.02 (t, 1H, -C**H**NH), 6.46 (s, 1H, -CO<sub>2</sub>N**H**), 4.30 (m, 1H, -C**H**(CH<sub>3</sub>)Br ), 3.60 (m, 2H, -NHC**H**<sub>2</sub>), 2.99 (t, 2H, -NHCH<sub>2</sub>C**H**<sub>2</sub>), 1.82 (d, 3H, -C**H**<sub>3</sub>(CH)Br).

# Synthesis of S-(1-((2-(1*H*-indol-3-yl)ethyl)amino)-1-oxopropan-2-yl) *O*-ethyl carbonodithioate (1), X21

The alkylating agent (**4**, 2.50 g, 8.47 mmol) was dissolved in 25 mL of dry ethanol and potassium *O*-ethyl xanthate (1.81 g, 11.23 mmol) was added and left stirring for 30 hours. A white precipitate was filtered off and the filtrate concentrated *via* rotary evaporation under vacuum. The viscous solution was diluted with 250 mL of diethyl ether. A white precipitate had formed, which was removed after washing with water (4x50 mL) as it dissolved in the aqueous phase. The organic phase was dried over anhydrous magnesium sulphate and the solvents evaporated by rotary evaporation under high vacuum. 2.91 g of a highly viscous orange product was obtained and purified *via* column chromatography using an initial solvent system of ethyl acetate: pentane in a 60:40 v/v ratio, while gradually increasing the percentage of ethyl acetate. The solvents were then evaporated under high vacuum, which resulted in 2.18 g of an orange yellow product, which was identified as the final product *via* <sup>1</sup>H-NMR spectroscopy. Yield = 2.18 g, 6.48 mmol, 76%.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>,  $\delta$ , ppm ): 8.18 (s, 1H, -CHNH), 7.60 (m, 1H, ArH), 7.37 (m, 1H, ArH), 7.24 – 7.07 (m, 2H, ArH), 7.01 (d, 1H, -CHNH), 6.39 (s, 1H, -CO<sub>2</sub>NH), 4.50 (m, 2H, (-OCH<sub>2</sub>CH<sub>3</sub>), 4.30 (m, 1H, -CH(CH<sub>3</sub>)S), 3.60 (m, 2H, -NHCH<sub>2</sub>), 2.97 (t, 2H, -NHCH<sub>2</sub>CH<sub>2</sub>), 1.50 (m, 3H, -CH<sub>3</sub>(CH)S), 1.33 (t, *3H*, -CHCH<sub>3</sub>).



# Synthesis of γ-Benzyl-L-glutamate (BLG) *N*-carboxyanhydride (NCA)

Scheme S2 Synthesis of the NCA of γ-benzyl-L-glutamate (BLG)

#### Synthesis of γ-Benzyl-L-glutamate (6)

The general procedure used for the synthesis of  $\gamma$ -benzyl-L-glutamate was adapted from the method used by Blout *et al.*<sup>1</sup> L-glutamic acid (100 g, 0.680 mol) and 182 mL of 48% hydrobromic acid were added to 670 mL of benzyl alcohol under stirring. The mixture was immersed into a preheated oil bath of 65 °C and kept under continuous stirring until all the glutamic acid was in solution (ca. 30 min). Once the solution had turned clear, it was rapidly cooled to ca. 30 – 40 °C. A solution of 200 mL pyridine in 1350 mL of 95% ethanol was added to the clear solution after which precipitation occurred upon further cooling. The reaction mixture was kept at 0 – 4 °C for a further 12 hours to ensure complete

precipitation of the product. The precipitate was filtered off and washed with ethanol and diethyl ether and dried under high vacuum at room temperature.

The product was recrystallized from 5% aqueous ethanol (keeping the temperature at 65 °C to prevent thermal decomposition) with a sufficient amount of sodium bicarbonate to keep the pH of the mixture at 7. The solution was kept at high temperature for a minimum amount of time to prevent decomposition into benzyl alcohol and glutamic acid. The solution was rapidly cooled and kept at 0-4 °C for a further 12 hours to ensure complete precipitation. The product was isolated *via* filtration and subsequently washed with water (pH adjusted to 7 with sodium bicarbonate), distilled water and then dispersed in ethanol. The product was isolated *via* filtration, washed with diethyl ether and dried under high vacuum at room temperature. The product was stored under refrigeration. Yield: 36.7 g, 155 mmol, 23%.

<sup>1</sup>H NMR (300 MHz, TFA, δ, ppm) 11.63 (s, 1H, OH), 7.47 – 7.32 (m, J = 9.3 Hz, 5H, ArH), 5.31 (s, 2H, -OCH<sub>2</sub>), 4.56 (t, J = 6.1 Hz, 1H, (-(CO)CH(NH<sub>2</sub>)), 2.98 (t, J = 6.6 Hz, 2H, -COCH<sub>2</sub>), 2.73 – 2.46 (m, 2H, CH2). <sup>13</sup>C NMR (400 MHz, TFA, δ, ppm) 176.12 (CHCO<sub>2</sub>H), 172.46 (CH<sub>2</sub>CO<sub>2</sub>), 133.67 (Ar), 128.63 (Ar), 128.30 (Ar), 127.82 (Ar), 68.83 (CH<sub>2</sub>O), 53.38 (CH), 30.11 (CH<sub>2</sub>CO), 24.27 (CH<sub>2</sub>CH).

# Synthesis of the BLG NCA (8)

γ-Benzyl-L-glutamate (10 g, 0.042 mol) was dissolved in 100 mL of anhydrous THF and the mixture warmed to 50 °C under stirring. One third of an equivalent triphosgene (4.98 g, 0.0168 mol, **7**) was added in a single aliquot after which the reaction mixture was left under reflux while venting into a 1 M NaOH trap. The solution became homogenous after 1 hour, but was left for three hours after which the reaction mixture was poured into 300 mL of *n*-pentane. Crystals formed immediately, but the suspension was left overnight at -10 °C to ensure complete precipitation. The NCA was recrystallized from THF/*n*-pentane (1:3 *v/v*) and subsequently stored in the refrigerator under P<sub>2</sub>O<sub>5</sub>. Yield: 8.4 g, 31.9 mmol, 76%

<sup>1</sup>H NMR (400 MHz, CDCL<sub>3</sub>,  $\delta$ , ppm): 7.36 (m, 5H, ArH ), 6.51 (s, 1H, (NH)), 5.14 (s, 2H, OCH<sub>2</sub>), 4.37 (t, *J* = 6.1 Hz, 1H, CH), 2.60 (t, *J* = 6.8 Hz, 2H, COCH<sub>2</sub>), 2.36 – 2.03 (m, 2H, CH2). <sup>13</sup>C NMR (400 MHz, CDCL<sub>3</sub>,  $\delta$ , ppm) 171.42 (CHCO<sub>2</sub>), 168.39 (CH<sub>2</sub>CO<sub>2</sub>), 150.89 (NHCO<sub>2</sub>), 134.24 (Ar), 127.77 (Ar), 127.64 (Ar), 127.43 (Ar), 66.16 (CH<sub>2</sub>O), 55.97 (CH), 28.88 (CH<sub>2</sub>CO), 25.95 (CH<sub>2</sub>CH).

#### Preparation of $\omega$ -aldehyde end-functional PVP

#### Quantification of the chain end-functionality:

<sup>1</sup>H-NMR spectroscopy was used to quantitatively determine the extent of the end-group conversion to the  $\omega$ -aldehyde end-functional PVP.

<sup>1</sup>H-NMR spectroscopy confirmed the conversion of the xanthate to the hydroxyl functionality. The hydroxyl functionality appears as a broad peak in the range of  $\delta$  = 5.2 – 5.5 ppm (**B**) along with the disappearance of the xanthate's two-proton methylene signal, **A** (Figure S1).



Figure S1 <sup>1</sup>H-NMR spectra of PVP in CDCl<sub>3</sub> before and after being heated in aqueous solution (pH = 4.5) at 40 °C for 20 hours.

After hydrolysis of the xanthate end-groups, the resulting polymer was heated at 120 °C for 20 hours under vacuum. The final product was aldehyde functionalized PVP, peak **C** in Figure S2. The end-group conversion to the aldehyde was calculated by referencing the subsequent integration peaks to the methyl protons (3H, see Figure S3, **k**) found at the  $\alpha$ -chain-end, signal **D** (Figure S2,  $\delta$  = 1.07 ppm) (the assumption was made that every chain was initiated *via* RAFT polymerization and therefore has an aromatic  $\alpha$ -chain-end). Also, the ratio of alpha and omega functionalities remained constant and were not affected by the reaction conditions. This was verified by the constant ratio of signal D to the

combined signals of the repeat unit (2.8 - 4.1 ppm). However, the difficulty in quantifying polymers due to the broadness of polymeric peaks should still be stressed.



Figure S2 <sup>1</sup>H-NMR spectrum of PVP in CDCl<sub>3</sub> after being hydrolyzed and subsequently heated for 20 hours at 120 °C under vacuum.



Figure S 3 PVP structure after the polymerization of NVP with the RAFT CTA X21 at 60 °C with AIBN as the initiator.

#### Optimization of $\omega$ -aldehyde end-group synthesis

The hydrolytic stability of the thiocarbonyl thio moiety from the RAFT agent is known to be pH-dependent (Figure S4).<sup>2</sup> The influence of pH was investigated by varying the aqueous conditions during the initial hydrolysis step. Conditions were investigated to optimize the conversion of xanthate end-groups to aldehyde end-groups as well as decrease the fraction of unsaturated chain-ends formed as a by-product due to thermal elimination of the xanthate



Figure S4 <sup>1</sup>H-NMR spectra of PVP-Xanthate (CDCl<sub>3</sub>) after hydrolysis under varying aqueous conditions. The pH of the aqueous solutions was 3 (top), 2 (middle) and 1 (bottom) respectively.

#### Cysteine end-functionalized PBLG

The removal of the protecting groups for the preparation of the cysteine endfunctionalized PBLG is illustrated in Scheme S3. The initial deprotection step (A) entails the removal of the Fmoc group resulting in free primary amine and the protected cysteine. The subsequent step (B) cleaves the Acm group to produce the free thiol and subsequently the terminal cysteine.



# Scheme S3 Deprotection steps of the Fmoc-cys(Acm) end-functional PBLG resulting in the terminal cysteine moiety.

After the peptide coupling and initial deprotection step as well as the subsequent purification of the product, end-group analysis was done using MALDI-ToF-MS. This clearly confirmed that aside from the presence of a small fraction of pyroglutamate end-group structures, the PBLG polypeptide did contain the protected terminal cysteine functionality (Figure S5).

The main distribution, peak **C**, represents PBLG with the protected cysteine endgroups (**9**) (DP = 28). In addition, there is a secondary distribution (peak **B**) due to intramolecular ring-closure resulting in the terminal pyroglutamate chain-end. Furthermore, the Kaiser test<sup>3</sup> was performed for the coupling reaction of the Fmoc-L-Cys(Acm) group with the primary amine functionality on PBLG. The test indicated that no free amines were present and that coupling had thus taken place.



Figure S5 MALDI-ToF-MS of PBLG after the coupling reaction with Fmoc-Cys(Acm) group.

Table S1 shows all the structural assignments for the MALDI-ToF-MS data obtained in the synthesis of PBLG as well as the protection/deprotection steps. The products obtained after the deprotection of the amine functionality (removal of the Fmoc group, **10**) have been identified (MALDI-ToF-MS spectrum not shown).

Experimentally, the low MW polymer in the distribution agreed well with the calculated values. Due to calibration inaccuracies, the high MW polypeptides constantly deviated from the isotopic distribution calculations by roughly 4 m/z units.

Table S1End-group characterization of PBLG before and after the peptide<br/>coupling reaction and subsequent deprotection steps. Structural<br/>assignments were achieved via MALDI-ToF-MS.

Peak	Monoisotop (+K⁺) <sup>a</sup> Expt.	ic mass <sup>b</sup> Theo.	Structure	°n
В*	6610.36	6613.97		29
С	6678.91	6681.09	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} $	28

<sup>a</sup>Experimentally determined number average molar mass, <sup>b</sup>theoretical molar mass, <sup>c</sup>degree of polymerization



# TEM images of PVP-b-PBGL nanoparticles









# Figure S6 TEM images indicating particle sizes of $PVP_{90}$ -*b*-PBLG<sub>41</sub> (A), $PVP_{90}$ -*b*-PBLG<sub>54</sub> (B), $PVP_{27}$ -*b*-PBLG<sub>54</sub> (C), $PVP_{225}$ -*b*-PBLG<sub>54</sub> (D) and $PBLG_{54}$ -*b*-PVP<sub>62</sub>-*b*-PBLG<sub>54</sub> (E).

#### Effect of pH on particle size

The dependence of the particle size on pH was investigated for  $PVP_{27}$ -*b*-PBLG<sub>54</sub> because of the narrow particle size distributions measured by DLS. The micelles were dialyzed against a sodium acetate buffer system, pH 5.4 for 48 hours and 168 hours. The diameters were measured with DLS (Figure S7) after 48 hours.





The <sup>1</sup>H-NMR spectra in Figure S8 further illustrate the extent of the benzyl ester hydrolysis. Peaks **A** and **B** represent the aromatic benzyl protons and the benzylic methylene protons on the PBLG backbone, respectively. Peaks **C** correspond to the backbone protons of the PVP repeat unit. The bottom spectrum represents the PVP-*b*-PBLG copolymer after being hydrolyzed in a sodium acetate buffer system of pH 5.4 for 48 hours.



Figure S8 <sup>1</sup>H-NMR spectra comparing PVP-*b*-PBLG in CDCl<sub>3</sub> before (bottom) and after (top) hydrolysis of the benzyl ester groups. The middle <sup>1</sup>H-NMR spectrum is the copolymer after hydrolysis in DMSO-d6.

The disappearance of peaks **A** and **B** in the top spectrum as well as the significant decrease in the middle spectrum is a clear indication of the hydrolysis of the benzyl ester groups. The top and middle spectra are identical samples, but in different solvents. It is interesting to note the complete disappearance of the benzylic proton signals in  $CDCl_3$ . This is most likely due to phase separation in the system, causing the hydrolyzed copolymer to self-assemble in  $CDCl_3$  which inhibits the mobility of the protons in the hydrophobic core and suppresses the signals.

#### CMC of PVP-b-PBGL

The CMC was determined for  $PVP_{90}$ -*b*-PBLG<sub>54</sub> *via* fluorescence spectroscopy with Nile Red as the fluorescent probe. This copolymer was formed quantitatively *i.e.* no starting material was present to influence the CMC calculation. The CMC was determined by plotting the maximum emission wavelength against the negative Log function of the micelle concentration. This was determined *via* the intersection of the extrapolated curves below and above the CMC (Figure S9).



Figure S9 The CMC determination for PVP<sub>90</sub>-*b*-PBLG<sub>54</sub> using fluorescence spectroscopy with Nile Red as the fluorescent probe.

The CMC for  $PVP_{90}$ -*b*-PBLG<sub>54</sub> was determined to be 6 µg/mL. This value compared well with CMCs determined for poly(ethylene glycol)-*b*-poly( $\gamma$ -benzyl-L-glutamate) block copolymers determined by Du *et al.*<sup>4</sup> that ranged from 1.37 – 2.14 µg/mL.

#### **References:**

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