Electronic Supplementary Information with:

**Polymer-protein conjugates from ω-aldehyde endfunctional poly(N-vinylpyrrolidone) synthesized via xanthate-mediated living radical polymerization**

by

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**Materials**

N-vinylpyrrolidone (NVP, Aldrich, 99 %) was dried over anhydrous magnesium sulfate and purified by distillation under reduced pressure. 2,2’-Azobisisobutyronitrile (AIBN) (Riedel de Haen) was recrystallized twice from methanol. Toluene, hexane and ethyl acetate (Merck) were distilled under reduced pressure. Tetrahydrofuran (KIMIX) was distilled over lithium aluminum hydride. Potassium O-ethyl dithiocarbonate (95 %, Merck), iodine, potassium iodide, cyclohexylamine, diethyl ether, anhydrous magnesium sulfate and lysozyme (Muramidase hydrochloride from Boehringer Manheim GmbH) were used as received. For column chromatography, silica gel (Fluka, particle size 0.063 – 0.2 mm, Brockmann 2-3) was used.

**Synthesis of the chain transfer agent (CTA)**

S-2-cyano-2-propyl O-ethyl xanthate was prepared in two steps according to the procedure by Zard and coworkers:[1]

1. **Synthesis of O,O-diethyl bisxanthate.** O,O-diethyl bisxanthate was prepared by a method derived from that of Shi et al.[2] Potassium O-ethyl xanthate (10.80 g, 6.7·10⁻² mol) was dissolved in distilled water (50 mL). A solution of iodine (3.3 g) and potassium iodide (1.7 g) in distilled water (50 mL) was added dropwise. The mixture was left to stir for 48 h. A yellow / orange oil separated, which was extracted with diethyl ether (4×50 mL). The combined ethereal fractions were extracted with distilled water (5×50 mL), dried over anhydrous magnesium sulfate and the solvents were evaporated under vacuum. 6.80 g of yellow oil was obtained (Yd = 82 %), purity > 97 % by NMR. ¹H-NMR (400 MHz, CDCl₃): δ[ppm] = 4.69, 4H, q, J=7.1 Hz, (C₂H₂); 1.42, 6H, t, J=7.1 Hz, (C₃H₃).

2. **Synthesis of S-2-cyano-2-propyl O-ethyl xanthate.** O,O-diethyl bisxanthate (6.76 g, 2.8·10⁻² mol) and AIBN (5.40 g, 3.3·10⁻² mol) were dissolved in toluene (40 mL). The solution was degassed with argon for 30 min then placed in an oil bath at 80 °C. After 2 h an additional portion of AIBN (3.6 g, 2.2·10⁻² mol) was added. The reaction was stopped after 7.5 h. Solvents were evaporated under vacuum. The product was purified by column chromatography using hexane : ethyl acetate 95:5 (v/v) as the eluent. Yield = 63 %, Purity > 97 % by NMR. ¹H-NMR (400 MHz, CDCl₃): δ[ppm] = 4.74, 2H, q, J=7.2 Hz (CH₂); 1.75, 6H, s, (C(CH₃)₂); 1.52, 3H, t, J=7.2 Hz (CH₂CH₃).

**Polymerization procedure**

In a typical polymerization experiment, AIBN (0.0442 g, 2.7·10⁻⁴ mol), the CTA S-1-cyanoethyl O-ethyl xanthate (0.422 g, 2.23·10⁻³ mol) and NVP (11.00 g, 9.90·10⁻² mol) and a magnetic stir-bar were placed in a pear-shaped 50 mL Schlenk flask. The polymerization mixture was degassed with a minimum of 3 freeze-pump-thaw cycles followed by the introduction of ultra-high purity argon. The flask was immersed in an oil bath preheated to 60 °C. After 6 h of stirring PVP with xanthate end-groups (PVP 1-X) was isolated by precipitation from diethyl ether. The polymer was redissolved in dichloromethane and precipitated a second time from diethyl ether. Isolated yield = 46 %. \(M_n,SEC = 2710 \text{ g·mol}^{-1}\) (PMMA equivalents in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), \(PDI = 1.21\).
The same procedure was applied for preparing PVPs with different $M_n$ in the range 2000 – 18 000 g·mol$^{-1}$ by varying the concentration of the CTA. The ratio $[\text{CTA}]:[\text{AIBN}] = 8:1$ was kept constant in all polymerizations.

**PVP end-group modification**

**Preparation of PVP with hydroxyl end-group**

PVP$_1$-$X$ (1.00 g) was dissolved in distilled water (20 mL) and the solution (pH = 5) was heated at 40 °C for 16 h. The solution was dialyzed against distilled water using SnakeSkin® pleated dialysis tubing (Pierce, molecular weight cut-off = 3500 g·mol$^{-1}$ dextran equivalents) for 24 h at room temperature. The polymer with hydroxyl end-group (PVP$_1$-OH) was recovered by freeze-drying ($M_n,\text{SEC} = 2780$ g·mol$^{-1}$ (PMMA equivalents in HFIP), PDI = 1.20).

**Preparation of PVP with aldehyde end-group**

PVP$_1$-OH (0.50 g of white powder) was placed in a vacuum oven and heated at 120-130 °C (~1 mbar) for 20 h to yield PVP with aldehyde end-group (PVP-CHO) ($M_n,\text{SEC} = 2640$ g·mol$^{-1}$ (PMMA equivalents in HFIP), PDI = 1.21).

**Preparation of PVP with thiol end-group**

PVP$_1$-$X$ (0.30 g) was dissolved in tetrahydrofuran (2.5 mL). The solution was degassed by bubbling ultra-high purity argon and left to stir for 16 h at room temperature. The polymer with thiol end-groups (PVP-SH) was recovered by precipitation from diethyl ether ($M_n,\text{SEC} = 4300$ g·mol$^{-1}$ (PMMA equivalents in HFIP), PDI = 1.26). Dichloromethane was used in other experiments and may be preferable to tetrahydrofuran because tetrahydrofuran is not a good solvent for PVP with high molecular weight.

**Characterization**

$^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Varian 400 or 600 Varian $^{\text{Unity}}$ Inova spectrometer.

**Size-exclusion chromatography (SEC)**

The SEC set-up consisted of an eluent degasser (Alttech Elite), a gradient pump (Shimadzu, LC-10AD), an injector (Spark Holland, MIDAS), a two-column set (PSS, PFG Linear XL 7 μm, 8 x 300 mm, separation window $10^3 – 10^5$ Da), a column oven (Spark Holland, Mistral) at 40 °C, detectors in series: Dual Wavelength UV Detector (Waters, 2487); Light Scattering (RALS/LALS) and Viscometry (Viscotec, 270) and Differential Refractive-Index Detector (DRI) (Waters, 2414). The injection volume was 50 μL, the flow rate was 0.8 mL·min$^{-1}$. The eluent HFIP (Biosolve, AR-grade) with 0.02 M KTF added (potassium trifluoro acetate, 3.0 g·L$^{-1}$, Fluka 91702) was redistilled after use. A short silica column was placed after the pump to catch free fluoride, possibly present in HFIP. A particle filter 0.2 μm PTFE was placed between columns and UV detector to prevent small particles entering the LS detector. Data acquisition and processing were performed with Viscotec Omnisec 4.0 (all detectors) and Waters Empower 2.0 (UV and refractive index detectors). The calculated molecular weights were based on a calibration curve for poly(methyl methacrylate) standards (molecular weight range 650 - 1.5·10$^4$ g·mol$^{-1}$) of narrow polydispersity (Polymer Laboratories) in HFIP.

**Matrix-Assisted Laser-Desorption Ionization-Time-of-Flight-Mass Spectrometry (MALDI-ToF-MS)**

MALDI-ToF-MS measurements were performed on a Voyager DE-STR instrument (Applied Biosystems, Framingham, MA) equipped with a 337-nm nitrogen laser. Positive-ion spectra were acquired in the reflector mode. $\text{trans-}$2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]-malononitrile was used as the matrix. The matrix was dissolved in tetrahydrofuran at a concentration of approximately 40 mg·mL$^{-1}$. Potassium trifluoroacetate was used as the cationization agent and was
added to tetrahydrofuran at a concentration of 1 mg·mL⁻¹. The polymer sample was dissolved in tetrahydrofuran at a concentration of 2 mg·mL⁻¹. In a typical measurement, the matrix, cationization agent, and sample solutions were premixed in a 10:1:5 ratio. Approximately 0.5 μL of the obtained mixture was handspotted on the target plate and left to dry. For each spectrum, 1000 laser shots were accumulated. Data Explorer software (Applied Biosystems) was used for data interpretation.

**Gradient elution polymer chromatography (GPEC)**

GPEC was performed using a dual pump high performance liquid chromatography set up comprising the following units: Waters 2690 Separations Module (Alliance); Agilent 1100 series variable wavelength UV detector; PL-ELS 1000 detector. Data was recorded and processed using PSS WinGPC unity (Build 2019) software. A C18 grafted silica column was used (Luna RP C18 3 μm 150 × 4.60 mm, Phenomenex) at 30 ºC. The mobile phase composition was water (deionized, with 0.1 % formic acid):acetonitrile at a flow rate of 0.5 mL·min⁻¹. Samples were prepared in the same solvent composition as the mobile phase at the beginning of each elugram, at concentrations of 5 mg·mL⁻¹. The injection volume was 10 μL. A gradient was applied where the initial water content was 78 (vol) % (kept constant for 2 min after injection), decreased to 68 % over 10 min, kept constant at 68 % for 2 min, increased to 78 % within 1 min and kept at 78 % for 10 min before the next injection.

**Characterization results**

**Hydrolysis of PVP with xanthate end-group**

The aldehyde end-group on PVP samples apparent from ¹H- and ¹³C-NMR spectroscopy with the characteristic peak for the aldehyde carbon at 201 ppm seen in the ¹³C NMR spectrum (Figure S1). The structure was confirmed via ¹H/¹³C-heteronuclear single quantum coherence (HSQC)-NMR spectroscopy (Figure S2).

![13C-NMR spectrum](image)

**Figure S1**: ¹³C-NMR spectrum of poly(N-vinylpyrrolidone) with aldehyde end-group recorded in DMSO-­d₆. The polymerization product was precipitated in diethyl ether, dissolved in water, heated at 40 ºC for 16h, dialyzed, freeze-dried and heated at 120 ºC under vacuum for 20 h ($M_{n,SEC} = 2640$ g·mol⁻¹ (PMMA equivalents in HFIP), PDI = 1.21).
Figure S2: $^1$H/$^{13}$C-heteronuclear single quantum coherence NMR spectrum of poly(N-vinylpyrrolidone) with aldehyde end-group recorded in CDCl$_3$ (top) and enlargement in the region relevant to the aldehyde end-group signal (bottom).

The effect of pH on the end-groups obtained via hydrolysis can be observed indirectly via SEC. The increase in $M_n$ when hydrolysis was carried out at pH=12 indicates the formation of thiol end-groups and subsequent coupling of chains (Figure S).
Figure S3: Size-exclusion chromatograms of PVP with xanthate end-group (—), after hydrolysis at pH=10 (---) and pH=12 (····).