SMART METALLOPOLY(L-GLUTAMIC ACID) POLYMERS:
REVERSIBLE HELIX-TO-COIL TRANSITION AT NEUTRAL PH

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1. Materials and Methods

a. Materials

ZnSO$_4$.7H$_2$O, CuSO$_4$, NiSO$_4$.6H$_2$O, CoSO$_4$.H$_2$O, CaSO$_4$, sodium nitrilotriacetate (NTA), sodium iminodiacetate (IDA), glycine sodium salt hydrate (GLY), methanesulfonic acid (MSA), trifluoroacetic acid (TFA), anisole, H$_2$SO$_4$ and NaOH were purchased from Sigma-Aldrich and used without further purification. Diluted solutions were prepared with Milli-Q water. γ-benzyl-L-glutamate N-carboxyanhydride was purchased from Isochem. Propargylamine (98%) was double distilled before use. DMF was obtained from a Solvent Purification System (SPS) and freshly used for the polymerization.

b. Nuclear Magnetic Resonance (NMR)

NMR spectra were recorded on Bruker Avance spectrometer. Chemical shifts are reported relative to the deuterated solvents used (CDCl$_3$, D$_2$O).

c. pH measurements

The pH of every sample were measured with a Mettler Toledo SevenCompact™ S220 pH-meter, calibrated with Mettler Toledo buffer solutions between pH = 4 and pH = 10.

d. Circular Dichroism (CD)

The measurements were performed on a JASCO J-815 spectropolarimeter between 195 nm and 260 nm (far-UV), by using a quartz cell of 1 cm path length, at the desired temperature (20 °C for standard measurements, or a temperature gradient between 10 °C and 80 °C for special measurements). The measure parameters were optimized as follows: sensitivity between 5 and 200 mdeg, 0.01 mdeg resolution, 8 seconds response time (Digital Integration Time), 1 nm bandwidth and 10 nm/ min scanning rate. The polypeptide solutions were diluted
with Milli-Q water. The pH of the solutions was adjusted either by using H$_2$SO$_4$ or NaOH aqueous solutions (0.1 M).

2. Synthesis and characterization of poly(L-glutamic acid) 1-4:

\[ \text{Scheme 1. Poly}(L\text{-glutamic acid}) \text{ 1-4 preparation in 2 steps} \]

*Synthesis of α-propargyl PBLG.* In this work, propargylamine was used to initiate the ring-opening polymerization of γ-benzyl-L-glutamate-N-carboxyanhydride in DMF (see scheme 1). This initiation step would permit to produce a clickable poly(γ-benzyl-L-glutamate) block that can be further used for chemical ligation.\textsuperscript{1} The NCA monomer of γ-benzyl-L-glutamate (BLG-NCA, 2 g, 7.6 mmol) was weighed in a glovebox under argon, introduced in a flame-dried schlenk, and dissolved with 4 mL of anhydrous DMF. The solution was stirred for 10 min, and propargylamine (for instance, 16 μL for PGA 1, 0.25 mmol) was added with an argon purged syringe. The solution was stirred for 3 days at room temperature under argon. The polymer was then recovered by precipitation in diethylether and dried under high vacuum, analyzed by $^1$H NMR (CDCl$_3$ + 15% trifluoroacetic acid). Yield: 81%. Molar masses were first determined by $^1$H NMR using the intensity of methylene protons of the initiator at 3.9 ppm and the intensity of the PBLG at 5.1 ppm. Representative $^1$H-NMR of the polypeptide backbone (400 MHz, $\delta$, ppm): 2.13 (m, 2H, CH$_2$), 2.59 (t, 2H, CH$_2$, J = 7.1 Hz), 4.37 (t, 1H, CH, J = 6.6 Hz), 5.13 (s, 2H, CH$_2$O), 6.75 (s, 1H, NH), 7.35 (m, 5H, ArH). Molar masses were then determined by SEC in DMF/LiBr (1%) using an Acquity Advanced
Polymer Chromatography System (Waters) equipped with an Acquity APC XT column for extended temperature organic-based separations (4.6 × 150 mm) and an Acquity Refractive Index detector. Calibration was performed by using polystyrene standards. Samples 1 to 4 (before deprotection, 5 mg.mL$^{-1}$) were dissolved in DMF and were run at a flow rate of 0.5 mL.min$^{-1}$ at 55 °C.

Figure S1. SEC traces of polypeptide 1-4 before benzyl deprotection. SEC in DMF (LiBr 0.1%)

*Synthesis of α-propargyl PGA.* Poly(γ-benzyl-L-glutamate) (PBLG, 500 mg, 2.28 mmol of BLG units) was dissolved in TFA (5 mL) and the reaction mixture was stirred at 0 °C (by using an ice bath). Then, anisole (1 mL) and MSA (5 mL) were added to the solution and the reaction mixture was stirred during 20 min at 0 °C followed by a stirring during 20 or more minutes at room temperature. The polymer was finally precipitated twice in Et$_2$O and collected by centrifugation. The precipitate was suspended in water (8 mL) and a saturated NaHCO$_3$ solution (9.5 mL). The resulting mixture was dialyzed against milliQwater (MWCO 1 kDa) and lyophilized to give sodium salt of the debenzylated polymer as a white solid. Yield: 340 mg (96 %). Representative $^1$H-NMR of the polypeptide backbone in D$_2$O (see figure S2, 400 MHz, δ, ppm): 1.94-1.85 (m, 2H, CH$_2$), 2.20 (m, 2H, CH$_2$), 4.24 (m, 1H, CH).
Molar masses were determined by $^1$H NMR by using the intensity of methylene protons of the initiator at 3.97 ppm and the intensity of the PGA at 4.34 ppm.

Figure S2. $^1$H-NMR of the PGA 1-4 in D$_2$O.
Molecular weights were precisely measured by size-exclusion chromatography with a multiangle light scattering detection (WYATT, Dawn Heleos) and a differential refractive index detector (WYATT, Optilab rEX) in order to determine average molecular weights and dispersity. PGA 1-4 were analyzed in 0.01M Na$_2$HPO$_4$ / 0.2 M NaNO$_3$, pH 9 using two columns from Shodex (OHpak SB-804 HQ) and a guard column. The dn/dc values were determined for each polymer with a differential refractive index detector (WYATT, Optilab rEX) in same buffer conditions as for the chromatographic elution.

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<th>Polymer</th>
<th>Theoretical Dp</th>
<th>Dp PBLG from $^1$H NMR</th>
<th>Mn PBLG from SEC (Dp)</th>
<th>Dp PBLG from SEC</th>
<th>Mn PGA from SEC (Dp)</th>
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*Table 1.* Molecular weights characterization of polypeptides 1-4 by $^1$H NMR and by SEC before benzyl deprotection (PBLG) or after benzyl deprotection (PGA).

3. **Circular Dichroism Spectroscopy**

3.a. **Generalities:**

The secondary structure of proteins/peptides (α-helix, β-sheet…) can be analyzed from the CD spectra in the range from 195 to 255 nm, corresponding to the peptide bond absorption. The secondary structure of PGA 1-4 was studied by CD spectroscopy using the following procedure: the final concentration (the concentration in the cuvette used for the CD analyses) was always 300 µM in monomer units, and generally 300 µM for the metallic species ($C_M/C_P$ =1, a metal ratio that corresponds to 1 equivalent of metal per carboxylic polymer side chain). The final volume in the cuvette was always 1 mL (pathlength of 10 mm). The pH was verified
and eventually adjusted once samples prepared and just before CD analysis. According to previous report, PGA structured in alpha helix present two minima both at 210 and 224 nm (figure S3). The “molar ellipticity” also called the “mean residue ellipticity” has been calculated as follow:

\[
[\Theta] = \frac{10 \times \Theta_{\text{obs}}}{l \times c}
\]

\([\Theta]\) is expressed in deg.cm\(^2\).dmol\(^{-1}\) and \(\Theta_{\text{obs}}\) was the observed ellipticity in degrees (deg), \(l\) is the path length in dm, and \(c\) is the polypeptide concentration in mol/L. The \(\Theta_{\text{obs}}\) at 222 nm wavelengt was chosen as it is a recognized minimum for alpha helix structuring. It is to note that specific work done on PGA previously have showed that this minimum has been found at 224 nm and that the corresponding helicity strongly depends on the molecular weight and on the solubility.

**b. Preparation of samples and solutions for CD measurements:**

- Preparation of mother solutions used for the CD experiments:

  *PGA mother solution:* the polymer solution was always prepared the day before CD analysis. The mother solution of the polymer was prepared by dissolving the desired quantity of the polymer in Milli-Q water (polymer concentration: 12 mM in monomer units). For each experience, an aliquot of the desired volume was taken off from this solution and diluted in order to keep the final concentration at 300 \(\mu\)M in monomer units.

  *ZnSO\(_4\) mother solution:* a solution of ZnSO\(_4\) 30 mM was prepared by diluting the desired quantity of ZnSO\(_4\) in Milli-Q water. For each experience, an aliquot of the desired volume was taken off from this solution and diluted in order to keep the final concentration at 300 \(\mu\)M (for 1 equivalent per polymer side chain, \(C_M/C_P = 1\)).
**Ligand mother solution:** a solution of ligand 30 mM was prepared in Milli-Q water. For each experience, an aliquot of the desired volume was taken off from this solution and diluted in order to keep the final concentration at 300 μM (for 1 equivalent per polymer side chain \(C_M/C_P = 1\)).

- Preparation of solutions analyzed by CD:

  **Typical CD solution (Metal salt + PGA):** 25 μL of PGA mother solution (12 mM) was added in a tube, followed by 10 μL (1 equivalent/polymer carboxylic unit \(C_M/C_P = 1\)) of a metal salt solution (30 mM), and 950 μL of Milli-Q water eventually mixed with few uL of aqueous solutions of NaOH 0.1 M and or \(H_2SO_4\) 0.1 M if necessary. The volume was completed to 1mL final volume with Milli-Q water. The samples were always prepared the day before analyses.

  **Typical CD solution (Metal salt + PGA) for the screening of the stoichiometry by CD:** 25 μL of 12 mM PGA solution was added in a tube, followed by the desired quantity of a 30 mM solution of \(ZnSO_4\) (2.5 μL for 0.25 equivalent, 5 μL for 0.5 equivalent, 7.5 μL for 0.75 equivalent, 10.0 μL for 1 equivalent, and 950 μL of Milli-Q water eventually mixed with few uL of aqueous solutions of NaOH 0.1 M and or \(H_2SO_4\) 0.1 M if necessary, before completing the sample to 1mL final volume with Milli-Q water. The samples were always prepared the day before analyses.

  **Typical CD solution (Zn\(^{2+}\) salt + PGA) for \(T\) gradients measurements by CD:** 25 μL of PGA mother solution (12 mM) was added in a tube, followed by 10 μL (1 equivalent/polymer carboxylic unit \(C_M/C_P = 1\)) of a \(ZnSO_4\) mother solution (30 mM), and 950 μL of Milli-Q water eventually mixed with few uL of aqueous solutions of NaOH 0.1 M and or \(H_2SO_4\) 0.1 M if necessary. The volume was completed to 1 mL final volume with Milli-Q water.
water. The samples were always prepared the day before analyses. For acidic PGA measurements, the metal salt solution has been replaced by water. The temperature gradient was analyzed over a range from 10 °C to 80 °C with a scan speed of 10 °C / min. Before starting the measurements at each temperature; the sample was maintained at the desired temperature for 10 minutes in order to be sure that the analysis temperature inside the sample was reached.

*Typical CD solution (Metal salt + PGA + ligand) for destructuring study:* 25 µL of PGA mother solution (12 µM) was added in a tube, followed by 10 µL (1 equivalent/polymer carboxylic unit C_M/C_P = 1) of a ZnSO_4 mother solution (30 mM), and 940 µL of Milli-Q water. Then 10µL of a mother solution of ligand was added and the final mixture eventually mixed with few uL of aqueous solutions of NaOH 0.1 M and or H_2SO_4 0.1 M if necessary. The volume was completed to 1mL final volume with Milli-Q water. The samples were always prepared the day before analyses.
c. CD raw data:

**Figure S3.** CD spectra of an aqueous solution of PGA 1-4 at an adjusted pH of 7 (top) or 4 (down). [PGA] = 300 μM in monomer units.

**Figure S4.** Normalized CD spectra of an aqueous solution of PGA 1-4 at an adjusted pH of 4.
Figure S5. Normalized CD spectra of PGA 1-4 in aqueous solution at pH 7 once coordinated to Zn$^{2+}$ at a mixing ratio CM/CP = 1.

Figure S6 CD spectra of an aqueous solution of PGA 1-4 at a pH of 7 mixed with either ZnSO$_4$ or CaSO$_4$ at a mixing ratio CM/CP = 1. [PGA] = 300 μM. Spectra obtained after Ca$^{2+}$ addition evidenced a maximum at $\lambda = 218$ nm which is representative of a PGA coil structure. In marked contrast, spectra obtained after Zn$^{2+}$ addition evidenced a minimum at 225 nm which was indicative of an alpha helix structure.
Figure S7. CD spectra of an aqueous solution of PGA 1-4 at a pH of 7 mixed with ZnSO$_4$ at various mixing ratio $C_{\text{M}}/C_{\text{P}} =$ 0.25 to 1 (the darker curve, the higher ratio). $[\text{PGA}] = 300 \mu\text{M}$. Spectra obtained after Zn$^{2+}$ addition generally displayed
significant 210 nm/224 nm minima that are both indicative of an alpha helix PGA structure. Variation of the $\theta_{\text{obs}}$ value at the minimum could be attributed to metal induced helix aggregation.5

**Figure S8.** Temperature as a destructuring trigger of PGA 1-4 (pH 4, molar ellipticities calculated from $\theta_{\text{obs}}$ at 222 nm).

**Figure S9.** CD spectra of an aqueous solution of PGA 1-4 at a pH of 7 mixed with ZnSO$_4$ at a mixing ratio $C_M/C_P = 1$ and with NTA. [PGA] = 300 μM. Spectra obtained after Zn$^{2+}$ addition generally displayed significant 210 nm/224 nm minima that are both indicative of an alpha helix PGA structure. Addition of NTA fully reconvert the CD spectra in PGA coil structure as evidenced by the maximum at 218 nm.3
Figure S10. CD spectra of an aqueous solution of PGA 2 at a pH of 7 mixed with ZnSO₄ at a mixing ratio $C_\text{M}/C_\text{P} = 1$. [PGA] = 300 μM in monomer units. Spectra obtained after NTA addition displayed a maximum at 218 nm whatever the concentration in NaCl was.
Figure S11. CD spectra of an aqueous solution of PGA 1-4 at a pH of 7 mixed with ZnSO₄ at a mixing ratio Cₐ/Cₚ = 1 and with IDA (top) or GLY (down). [PGA] = 300 μM.
d. Zn$^{2+}$ coordination by NMR:

![13C-NMR spectra](image)

**Figure S12.** Left: $^{13}$C-NMR (100 MHz, carbonyl region) spectra of PGA 1 alone in D$_2$O. Right: $^{13}$C-NMR (carbonyl region) spectra of PGA 1 mixed with ZnNTA ($C_M/C_P = 1$) in D$_2$O. A slightly shift is observed for both polymer carbonyl moieties but, more important, a difference in the shape concerning the lateral chain carboxylic unit of PGA is observed upon addition of ZnNTA, indicating coordination of this moiety to the Zn$^{2+}$ metal center.

e. References