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Supporting Information

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Nanoribbons Self-assembled from Triblock Peptide Polymers and Coordination Polymers

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1. Experimental section

1.1 Materials

Preparation of 1:

Template DNA of the histidine containing charageable sequence(i. e., the middle block of **1**) $(GAGAGAGH)_2GA$ was produced by annealing two oligonucleotides and inserting the resulting double strand DNA into a pMTL23 derived vector. The charageable sequence was enlarged to the desired size $(GAGAGAGH)_{48}GA$ by restriction and directional ligation. The block was then combined with the neutral blocks (blocks with no chargeable groups, form random coils at all pH) to form the template gene which was transferred to the pPIC9 expression vector. *Pichia pastoris* transformed with pPIC9 was induced to produce the protein polymer in a fermenter at pH 5, essentially as described by Werten et al. [S1]. The secreted soluble protein was selectively precipitated from the fermentation supernatant by adding 258 g/kg ammonium sulfate(45 % saturation), incubating for 30 min at 21 °C and centrifugating for 20 min at 8000 rad/min and 4°C (Sorval, SLA1500). The polymer pellet was dissolved in 0.5 L 100 mM acetic acid from which the polymer was selectively precipitated by adding acetone to a final concentration of 50% (v/v). The pellet was dissolved in 300 mL 10 mM acetic acid and freeze-dried.

The salt containing freeze-dried product was resuspended in 100 mL 50 mM formic acid and dialyzed four times 18 h against 4L of 10 mM formic acid, after which the polymers were freeze-dried again and used for experiments. The final product were shown to have the expected length by mass spectrometry and N-terminal amino acid sequencing. Using the purified product as a standard, the amount of product present in the original fermentation broth was determined by densitometric analysis of the Coomassie-blue-stained product bands in a polyacrylamide gel. Several dilutions

were compared. The centrifuged, cell-free fermentation broth thus appeared to contain 6.1 g/l of ***1***, which is comparable to the highest yields of secreted heterologous protein published to date.[S2-4]

Other materials:

The zinc(II)-1,11-bis(2,6-dicarboxypyridin-4yloxy)-3,6,9-trioxaundecane metallo-supramolecules ($Zn\text{-}L_2EO_4$ complex), are is prepared according to literature.[S5] Ultra pure water was used throughout the experiments. pH was adjusted by HCl or NaOH.

1.2 Methods:

Dynamic light scattering (DLS). Light scattering measurements were performed with an ALV light scattering-apparatus, equipped with a 400 mW argon ion laser operating at a wavelength of 514.5 nm. A refractive index matching bath of filtered cis-decalin surrounded the cylindrical scattering cell, and the temperature was controlled at ± 0.5 ºC using a Haake C35 thermostat. Titrations were carried out using a Schott-Geräte computer controlled titration setup to control sequential addition of titrant and cell stirring. After every dosage, the laser light scattering intensity (I) at 90° were recorded.

Cryogenic transmission electronic microscope (Cryo-TEM). A few microliters of samples were placed on a bare copper TEM grid (Plano, 600 mesh), and the excess liquid was removed with filter paper. This sample was cryo-fixed by rapidly immersing into liquid ethane cooled to -170 to -180 °C in a cryo-box (Carl Zeiss NTS GmbH). The specimen was inserted into a cryo-transfer holder (CT3500, Gatan, Munich, Germany) and transferred to a Zeiss EM922 EFTEM (Zeiss NTS GmbH, Oberkochen, Germany). Examinations were carried out at temperatures around -180 °C. The TEM was operated at an acceleration voltage of 200 kV. Zero-loss filtered images were taken under reduced dose conditions (500-2000 e/nm^2). All images were recorded digitally by a bottom-mounted CCD camera system (UltraScan 1000, Gatan) and processed with a digital imaging processing system (Digital Micrograph 3.9 for GMS 1.4, Gatan).

Circular Dichroism (**CD**). Samples of pH 5.4 for CD measurements were prepared from stock solutions of mono **1** or a mixture of **1** and Zn-L_2EO_4. A dilution to 0.1g/L **1** for all samples was made in 20 mM PIPES buffer. For the measurement at pH 11, a salt free solution of **1** was prepared, and the solution was adjusted to pH 11 with 0.1 M NaOH, in which the concentration of **1** was also controlled at 0.1 g/L. The above solutions were permitted to age for more than 72 h in a refrigerator at 4 °C before measurement.

CD spectra were acquired on a CD Jasco J-715 Spectropolarimeter. Spectra were recorded in the far-UV region (190-260 nm) at 20 °C at a speed of 10nm/min and a sampling interval of 0.2 nm in a quartz cuvet with diameter 0.1 cm. 10 repeating measurements were made for all the samples. The background was subtracted for all the samples.

The obtained raw data of milidegrees (mdeg) were further transferred into ellipticities (Kdeg· $dmol^{-1}$· cm^2) by dividing the mdeg with the cuvett length pass and the molar concentration of the amino acids. The molar concentration of the amino acids is different from the molar concentration of the polypeptide, since one polypeptide has 802 amino acids in our case. In our experiments, the 0.1g/L polypeptide which has a molecular weight of 66135.3 g/mol gives an amino acid concentration of 0.0012 mol/L. The middle block has a concentration of 0.00059 mol/L, and the outer block is 0.00061 mol/L. With these concentrations, the new ellipticity values were obtained by using the following relations:

Ellipticity = mdeg/(mol/L)*cm = mdeg/(mol/$10^3$$cm^3$)*cm acid = 10^3mdeg/(mol/cm^2) = kdeg / (dmol/cm^2).

2. Figures

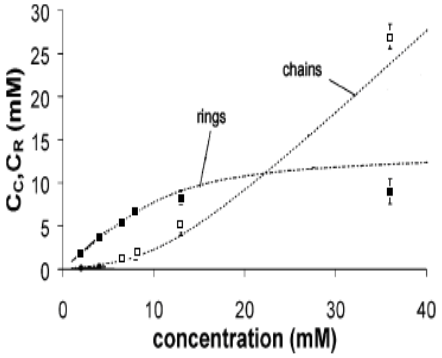

Figure S1: Conformation of the Zn-C4 coordination polymer at different concentrations

The concentrations of monomers in chains (□) and rings (■) as a function of the monomer concentration of Zn-L_2EO_4(1:1ratio) at 298K, determined from the integrals of the peaks in ^{1}H NMR spectra. The dotted lines refer to results of the theoretical model. See reference [S5] for the detailed information about this curve.

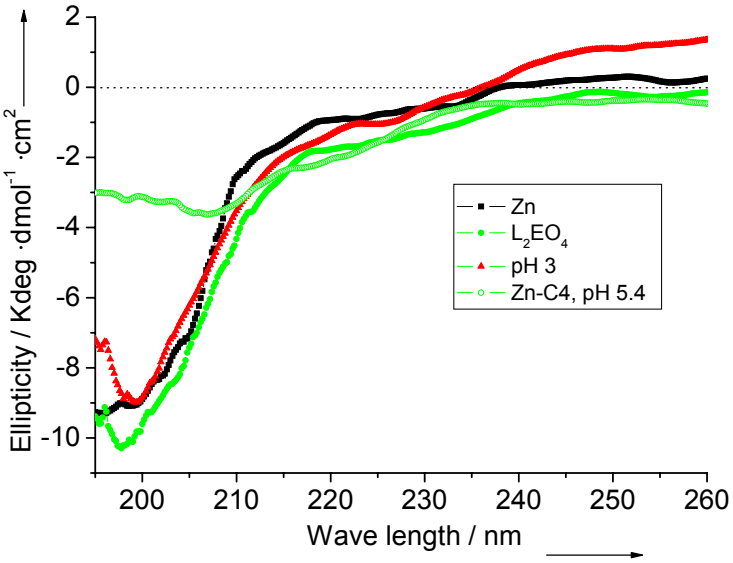

Figure S2: Comparison of the effect of different components on the CD spectrum of **1**. No contribution of the outer blocks were subtracted. It is clear from this figure that the conformation of **1** in the mixture of **1**/Zn^{2+} and**1**/L_2EO_4 is random coil. (pH 5.4) It is

obvious that zinc and pyridine dicarboxylic acid monoligands as single components have little effect on the secondary structure of **1**, although zinc ions may coordinate with histidine groups. Such observation is in good agreement with the DLS results that no obvious scattered light intensity was found when they were added separately to the solution of **1**.

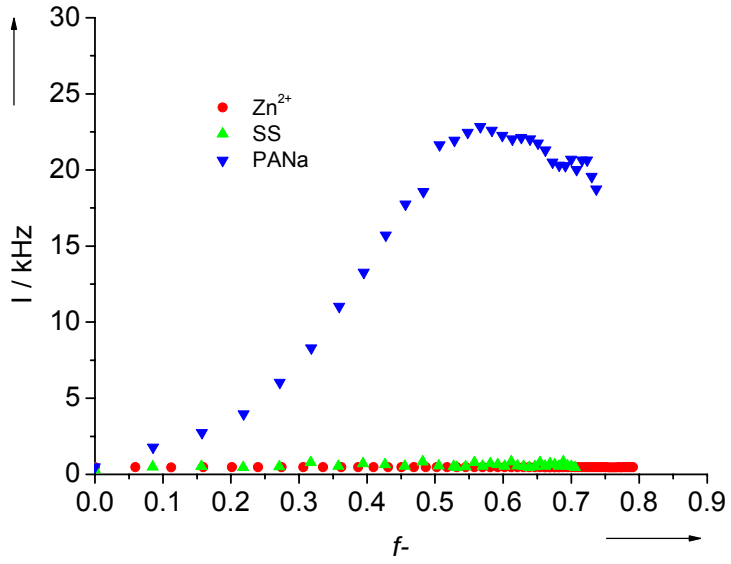

Figure S3: The scattered light intensity of 0. 18 mM **1** ([+], pH 5.4) affected by sodium succinate (SS), sodium polyacrylate($PANa_{48,}$ where 48 is the number of monomers per chain) and zinc ions. Three conclusions can be deduced from this figure: (i), Zn^{2+} can not result in self-assembly in combination with **1**; (ii), small negative organic ions which has similar size with the $Zn_2(L_2EO_4)_2^{2-}$ also cannot form self-assembly with **1**;(iii) polyions (PANa)with a negative charge density per chain similar to **1** results in formation of self-assembly. This also suggests that the small coordination rings of $Zn_2(L_2EO_4)_2^{2-}$ have transformed into long linear chains in the ribbons.

If the charge density per chain (the length of the charged block) of the oppositely charged blocks is mismatched, only soluble electrostatic complex other than self-assembly can be formed in the mixed system.[S6] By using coordination polymer Zn-

L_2EO_4, the length and the negative charges per chain become adaptable to the positively charged **1**, so that self-assembly, here nanoribbons, can always be formed.

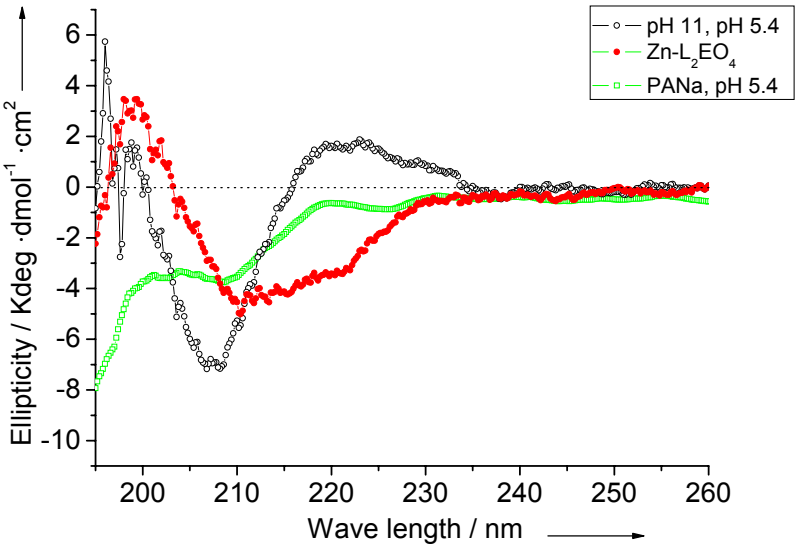

Figure S4: Comparison of the effects of pH, covalent polyelectrolyte PANa and coordination complex Zn-L_2EO_4 on the CD spectrum of **1**. The contribution of the outer blocks was subtracted from all the spectra. It is clear that increasing the pH induces predominant β-turns, while adding PANa results in a messy mixture of β-turns, helices, and probably some amount of β-sheets. Only the spectrum in the presence of coordination complex exhibits β-sheet features.

Figure S5: Gel formation in the mixture of **1** and Zn-L_2EO_4 at [**1**] = 7.9 g/L and pH 5.4 (with [+] = [-] = 5.8 mM, diameter of flask ~ 1 cm).

3. References

[S1]. M. W. T. Werten, F. A. de Wolf, *Appl. Environ. Microb*, **2005**,*71*,2310-2317.

[S2]. M. W. T. Werten, W. H. Wisselink, T. J. J. van den Bosch , E. C. de Bruin, F. A. de Wolf, *Protein Engineering*, **2001**, *14*, 447-454.

[S3]. M. W. T. Werten, T. J. J. van den Bosch, R.D. Wind, H. Mooibroek, F. A. de Wolf, *Yeast*, **1999**, *15*, 1087-1096.

[S4]. G. P. L. Cereghino, J. L. Cereghino, C. Ilgen, J. M. Cregg, *Current Opinion In Biotechnology*, **2002**, *13*, 329-332 .

[S5]. T. Vermonden, J. Van der Gucht, P. De Waard, A. T. M. Marcelis, N. A. M. Besseling, E. J. R. Sudhölter, G. J. Fleer, M. A. Cohen Stuart *Macromolecules*, **2003**, *36*, 7035-7044.

[S6]. M. A. Cohen Stuart, N. A. M. Besseling, R. G. Fokkink, *Langmuir*, **1998**, *14*, 6846-6849.