Supporting Information

Chain stiffness of elastin-like polypeptides

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Synthesis of elastin-like polypeptides. Five ELPs containing the sequence $(VPGVG)_n$, where n = 20, 30, 40, 60, and 120, were synthesized using plasmid reconstruction recursive directional ligation, as described elsewhere.[1] Each of the ELP genes was restricted from its parent plasmid, purified via gel purification (Qiagen Gel Purification Kit; Germantown, MD), and inserted into a modified pET-24a+ expression plasmid (Invitrogen; Carlsbad, CA).

The modified plasmid was constructed by replacing the NdeI – BamHI cloning region of the pET-24a+ vector with a synthetic oligomer cassette (Integrated DNA Technologies Inc.; Coralville, IA) encoding two opposing BseRI restriction sites flanked by an N-terminal methionine and C-terminal Phe-Cys (See Supplemental Figure 1). BseRI is a type IIs endonuclease, which unlike other commonly used restriction enzymes, cuts at a defined number of nucleotides from its recognition sequence. Hence two opposing restriction sites results in the self-excision of the interposing segment, leaving a linearized vector with 2-bp degenerate overhangs. 1.5 µg of the modified vector was thus linearized with 2U of BseRI at 37 °C overnight, treated with calf intestinal phosphatase for 1 h, and then ligated with the ELP gene containing compatible sticky ends. The product was transformed into chemically competent TOP10^{TM} cells (Invitrogen; Carlsbad, CA) and plated on TBDry agar plates (MO BIO Laboratories, Inc; Carlsbad, CA) supplemented with 45 µg/mL of kanamycin. The sequences were verified using DNA

sequencing. Following sequence confirmation, the ELP containing plasmid was transformed into BLR(DE3) (Novagen; Carlsbad, CA) expression cells.

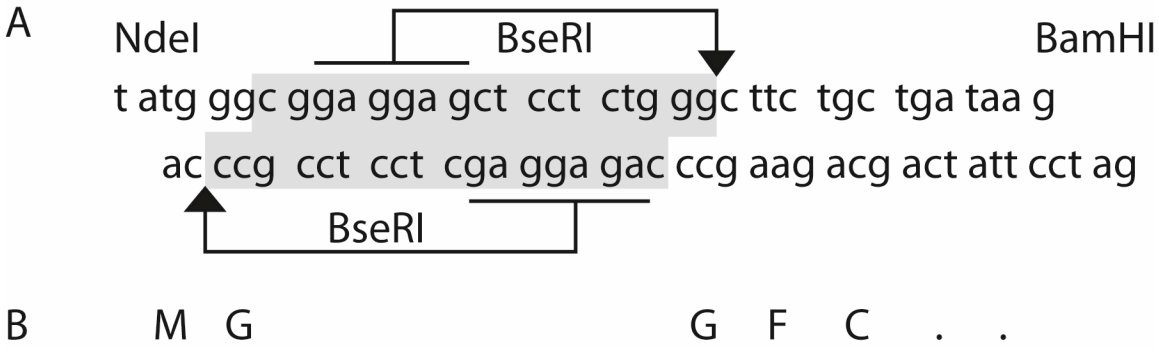

Figure S1: A) Oligomer cassette designed with NdeI and BamHI sticky ends. The BseRI recognition sequence (underlined) is spatially separated from its restriction site (arrow). Incubation with BseRI results in the self excision of the shaded area, allowing the seamless and directional integration of ELP with the degenerate 2-bp overhangs (GG and CC). **B)** The resultant construct contains an N-terminal methionine and a short C-terminal phenylalanine-cysteine sequence.

Expression and purification of ELP. Each ELP was expressed using a hyperexpression protocol, which relies on the leakiness of the T7 promoter.[2] 1 L cultures of TBDry supplemented with 45 µg/mL kanamycin were inoculated from 50 mL overnight cultures. The flask was incubated at 37 °C for 24 h and 210 rpm, following which the culture was centrifuged at 3,000 g for 10 min at 4 °C to pellet the cells. All ELP constructs were purified from cell lystate using the inverse thermal cycling (ITC) protocol as described elsewhere.[3] Briefly, the cell pellet was resuspended in a 1:1 ratio of PBS, placed on ice, and lysed via sonication (Sonicator 3000, Misonix; Farmingdale, NY). To remove nucleic acids contaminants, polyethyleneamine (PEI) was added to the mixture to a final concentration of 0.7 % w/v. Each round of ITC then proceeded as follows: the cell lysate was centrifuged at 14,000 g for 10 min at 4 °C to remove insoluble fractions. The supernatant was decanted, heated to 37 °C for 10 min, and then centrifuged at 11,000 g for 10 min at 30 °C in order to precipitate the insoluble ELP coacervate. In some cases, up to 3 M NaCl was added after heating in order to precipitate the ELP. The supernatant was again decanted, and the ELP pellet was resuspended in cold phosphate buffer. Typically, 3-5 rounds of ITC yielded > 95% purity as assessed by SDS-PAGE.

SDS-PAGE analysis. SDS-PAGE was performed in a Mini-Protean cell (Bio-Rad) using 4-20 % Tris-HCl precast gels. The gels were visualized with copper staining (0.5 M $CuCl_2$).

Light scattering characterization. Dynamic light scattering measurements were performed with an ALV-SP86 goniometer, an Uniphase HeNe laser (25 mW output power at 632.8 nm wavelength), an ALV/High QE APD avalanche diode fiberoptic detection system and an ALV-3000 correlator. The measurements were performed at 20 °C at the scattering angle of 30°. All ELP solutions were prepared in 20 mM NaCl at a concentration of 5 g/L. All solutions were filtered through anotop 0.02 µm filter (Whatman) followed by GHP 0.2 µm filter (Pall) into dust-free Suprasil cuvettes (20 mm diameter, Hellma, Mülheim).

Separate plots of the fits to the hydrodynamic radii as shown in Fig 2 of the main text

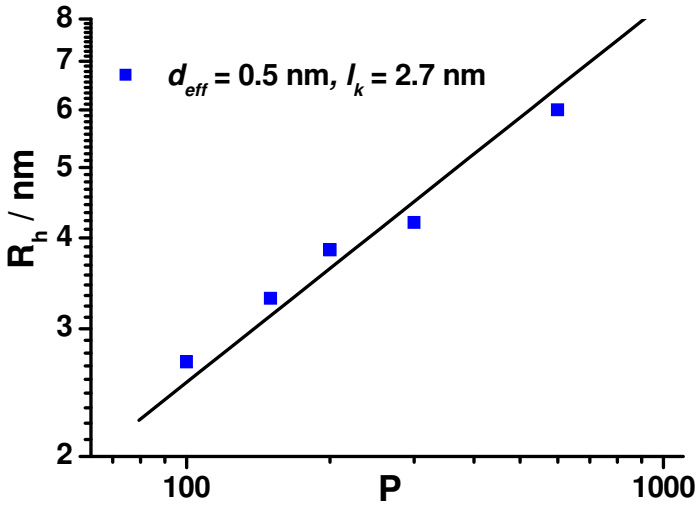

Figure S2: Double logarithmic plot of the hydrodynamic radius R_h vs. the number of peptide repeat units P. The red line represents the fit for d_{eff} = 0.5 nm, l_k = 2.7 nm to the data assuming a repeat unit length b = 0.365 nm.

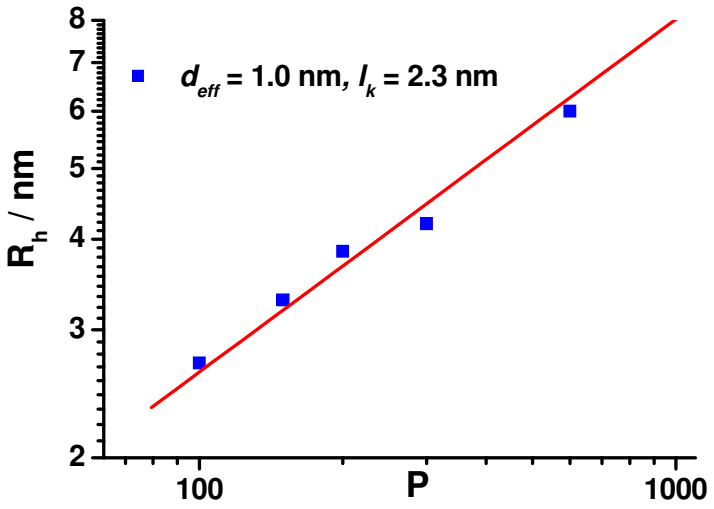

Figure S3: Double logarithmic plot of the hydrodynamic radius R_h vs. the number of peptide repeat units P. The red line represents the fit for d_{eff} = 1.0 nm, l_k = 2.3 nm to the data assuming a repeat unit length b = 0.365 nm.

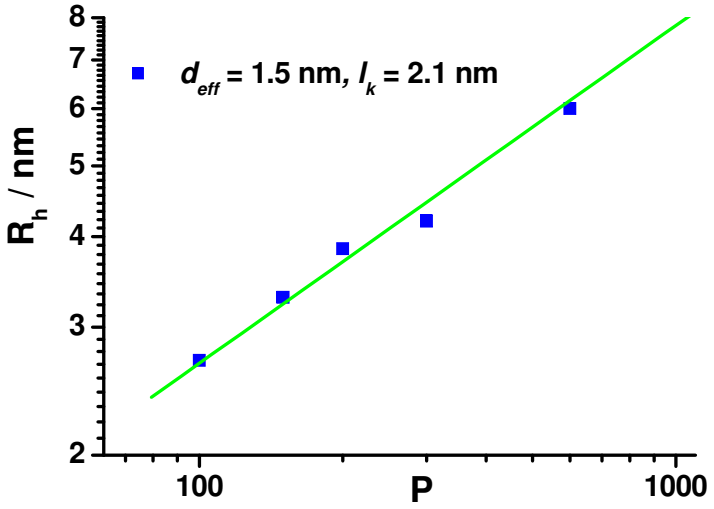

Figure S4: Double logarithmic plot of the hydrodynamic radius R_h vs. the number of peptide repeat units P. The green line represents the fit for d_{eff} = 1.5 nm, l_k = 2.1 nm to the data assuming a repeat unit length b = 0.365 nm.

Linear presentation of the data of Fig. 2 in the main text

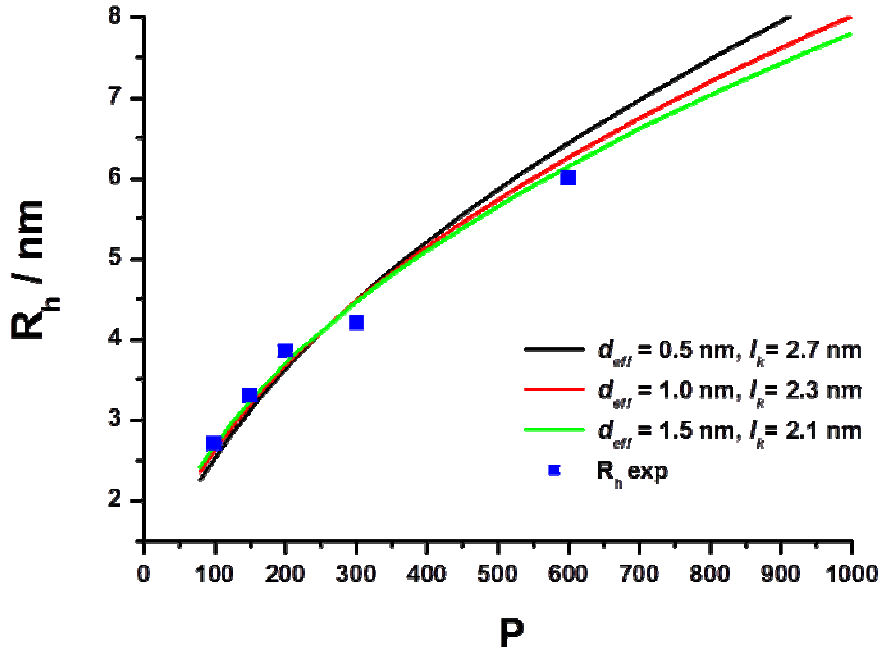

Fig. S5: Linear presentation of the hydrodynamic radius R_h vs. the number of peptide repeat units P. The lines represent the best fits to the data assuming a repeat unit length b = 0.365 nm. Black line: d_{eff} = 0.5 nm, l_k = 2.7 nm, red line: d_{eff} = 1.0 nm, l_k = 2.3 nm, green line: d_{eff} = 1.5 nm, l_k = 2.1 nm.

(1) McDaniel, J. R.; MacKay, J. A.; Quiroz, F. G.; Chilkoti, A. *Biomacromolecules* **2010**, *11*, 944-952.

(2) Guda, C.; Zhang, X.; McPherson, D. T.; Xu, J.; Cherry, J. H.; Urry, D. W.; Daniell, H. *Biotechnol. Lett.* **1995**, *17*, 745-750.

(3) Meyer, D. E.; Chilkoti, A. *Nat. Biotechnol.* **1999**, *17*, 1112-1115.