**Supporting Information**

Merging bioresponsive release of insulin-like growth factor I  
with 3D printable thermogelling hydrogels

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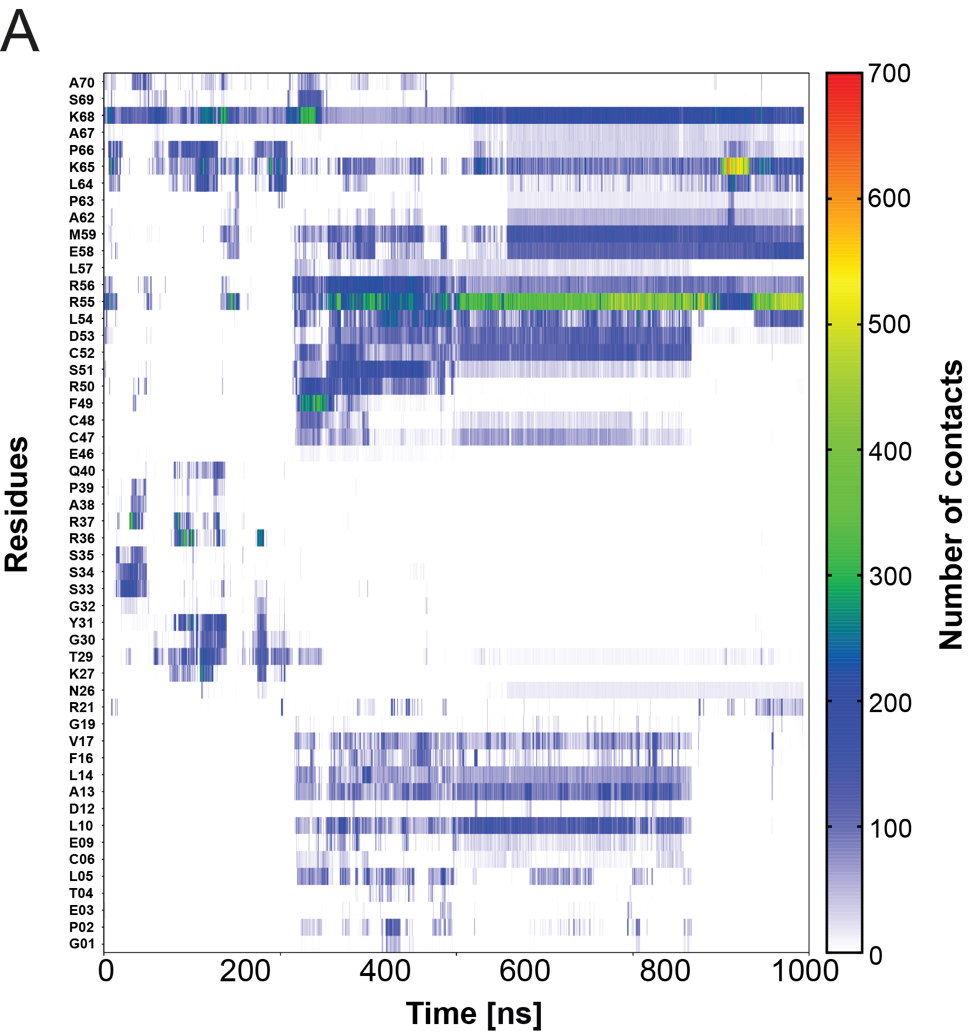
6 Soft Matter Chemistry, Department of Chemistry and Helsinki Institute of Sustainability Science, Faculty of Science, University of Helsinki, P.O. Box 55, 00014 Helsinki, Finland

Number of pages: S1–S14

Number of figures: S1–S14

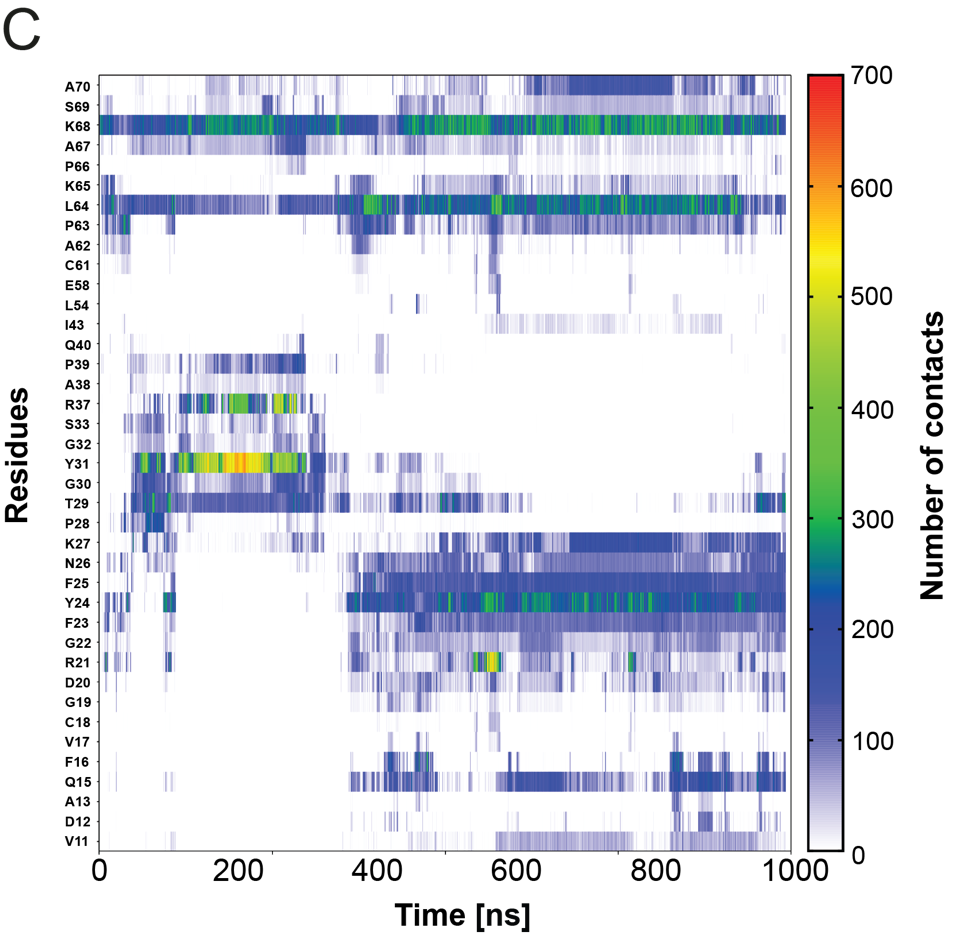
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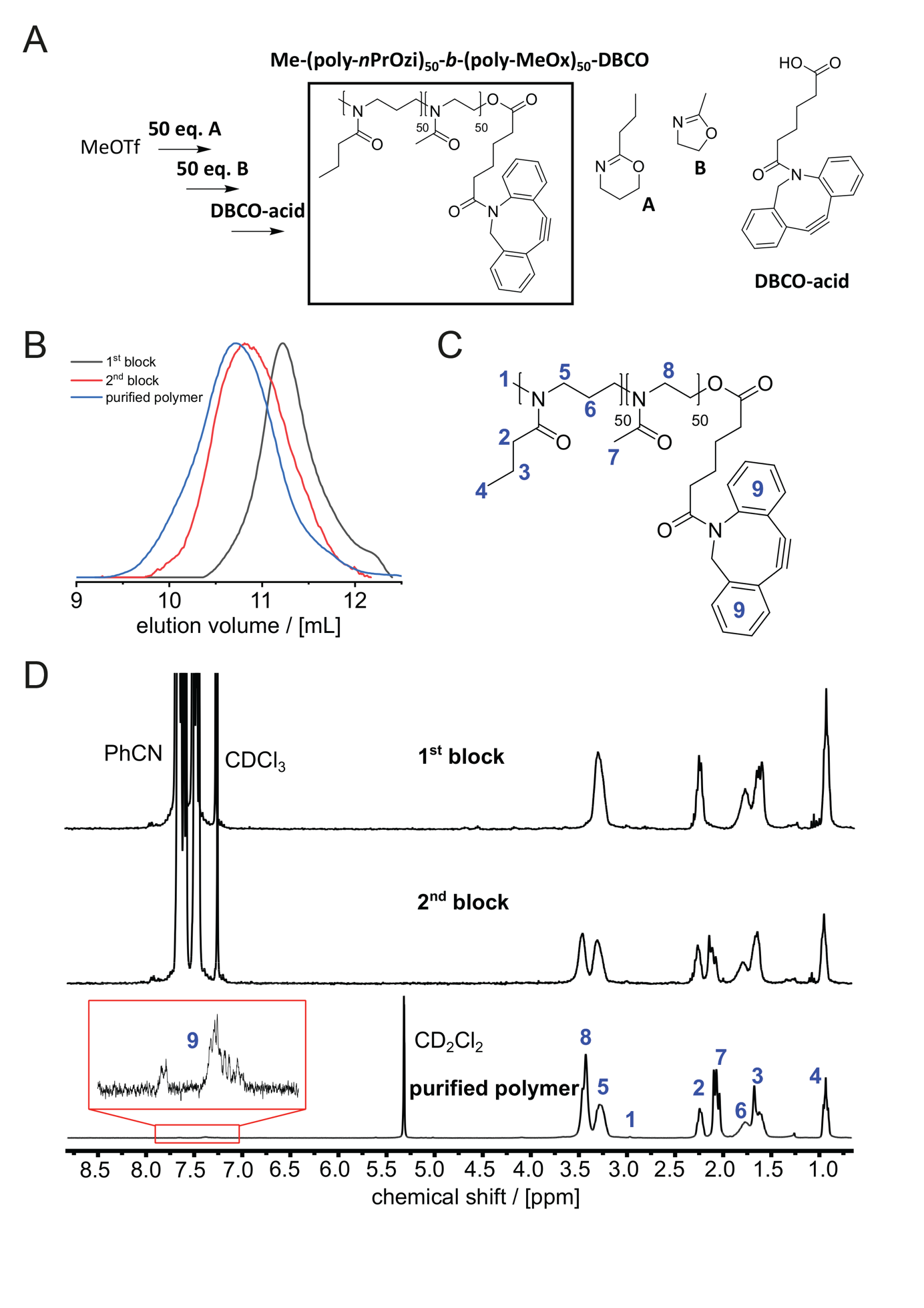


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**Figure S1:** (A) Number of contacts between PSL and IGF-I over the simulation time (run 2). The simulation time is shown as horizontal axis and the interacting IGF-I residues on the vertical axis. (B) Number of contacts between PSL and IGF-I over the simulation time (run 3). (C) Number of contacts between PSL and IGF-I over the simulation time (run 4). The simulation time is shown as horizontal axis and the interacting IGF-I residues on the vertical axis. The number of IGF-PSL contacts is color coded (see vertical scale bar on the right).



**Figure S2:** Synthesis and characterization of POx-b-POzi-DBCO (A) Schematic description of the synthesis of a thermoresponsive AB diblock copolymer (degree of polymerization: 100, block ratio: 1:1) comprising the thermoresponsive A block (poly-*n*PrOzi), the hydrophilic B block (poly-MeOx) and a DBCO omega functionality. (B) Following the reaction process via GPC. (C) Chemical structure of the AB diblock copolymer with the assignment of all relevant peaks in 1H NMR experiments. D) 1H NMR spectra after the 1st, 2nd block and the purified polymer.

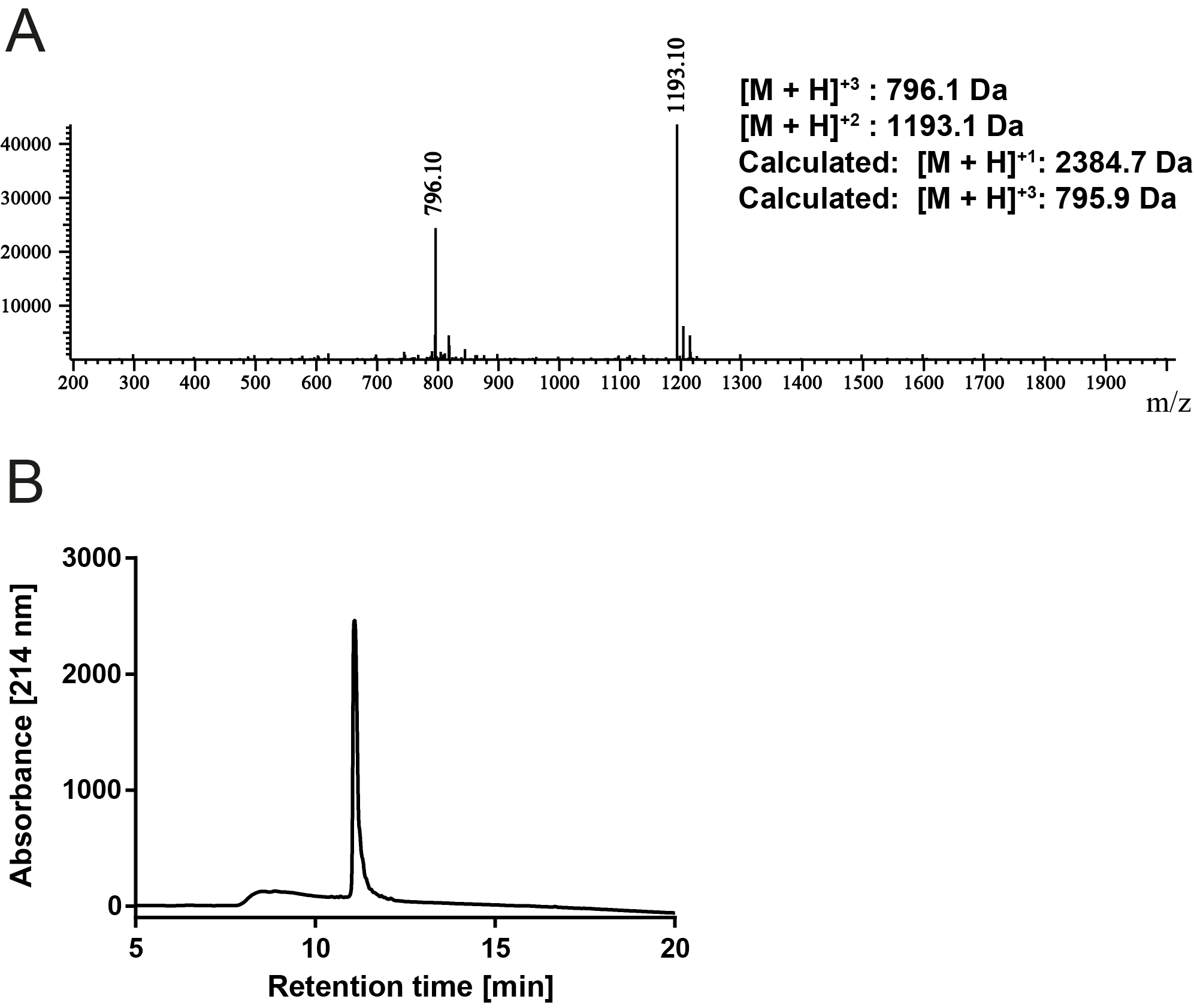


Figure S3: Characterization of PSL peptide. (A) ESI-MS analysis. (B) Purity of the peptide analyzed by HPLC.

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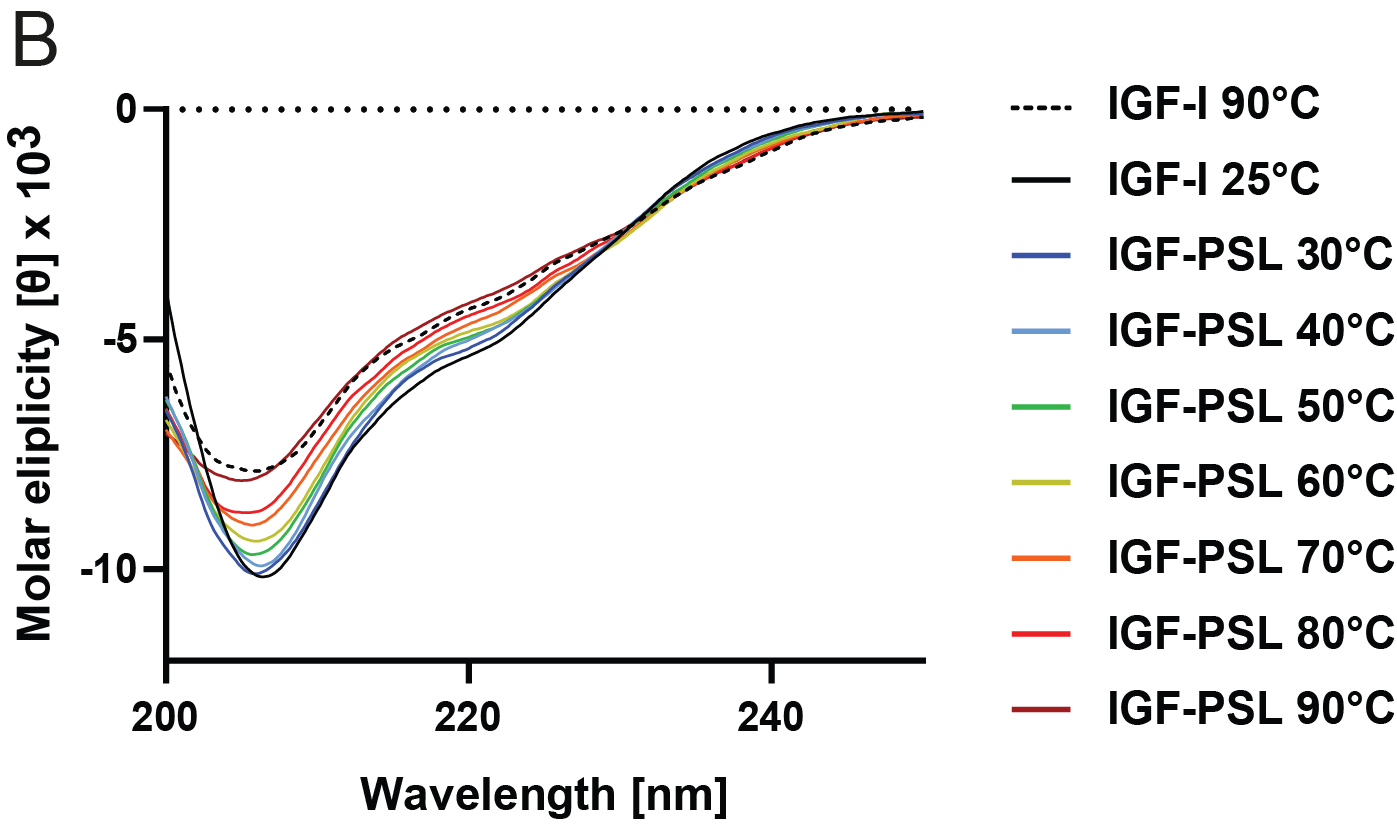
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**Figure S4:** MALDI-MS analysis of (A) IGF-I and (B) IGF-PSL.

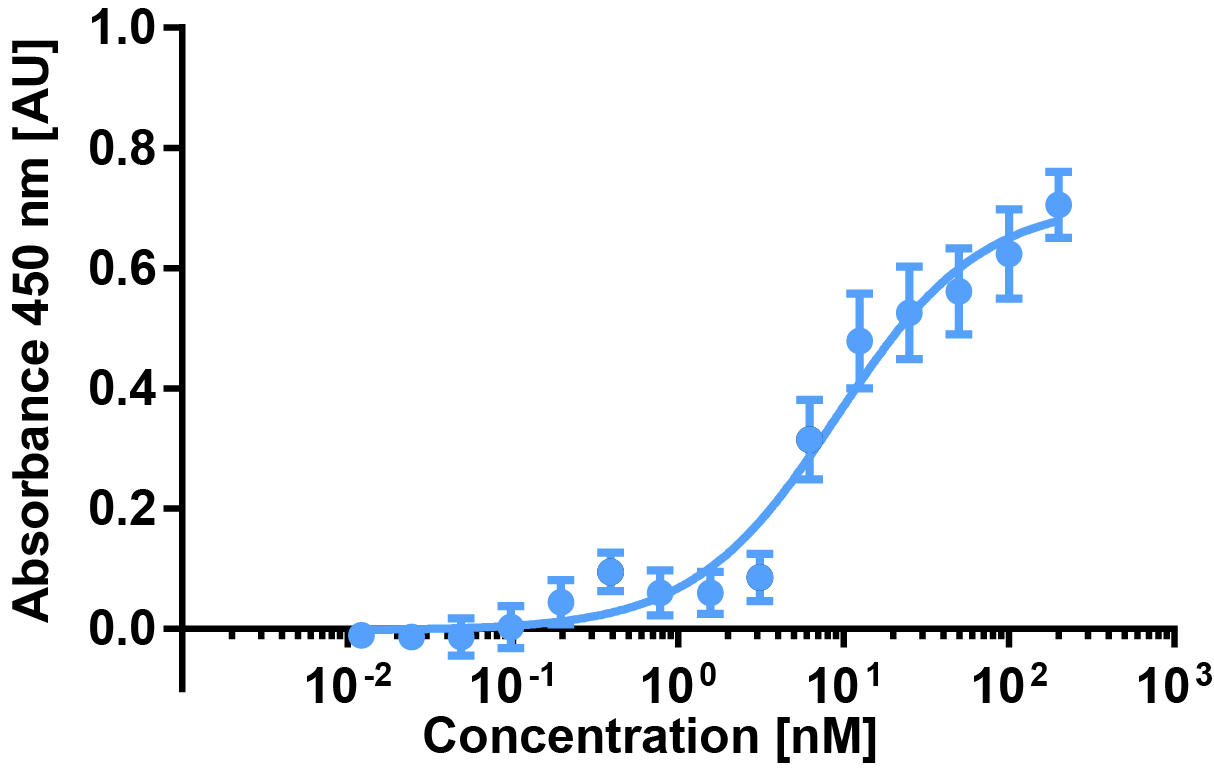
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Figure S5: HPLC chromatograms of IGF-PSL-POx-b-POzi incubated with 8 nm MMP-9 at different time points.



**Figure S6:** CD spectra of IGF-I (25 °C and 90 °C) and IGF-PSL at different temperatures ranging from 30 to 90 °C.



**Figure S7:** C2C12 myoblast proliferation assay with IGF-PSL (n=3 biological replicates and n=3 technical replicates).

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Figure S8: Bioactivity of IGF variants using western blot analysis of AKT and ERK phosphorylation in C2C12 myoblasts after exposure to (A) IGF-I, (B) IGF-PSL and (C) IGF-PSL-POx-b-POzi.

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**Figure S9:** Thermogelling properties of the different modified polymers. P-Ma and P-Fu at 4 °C and RT.

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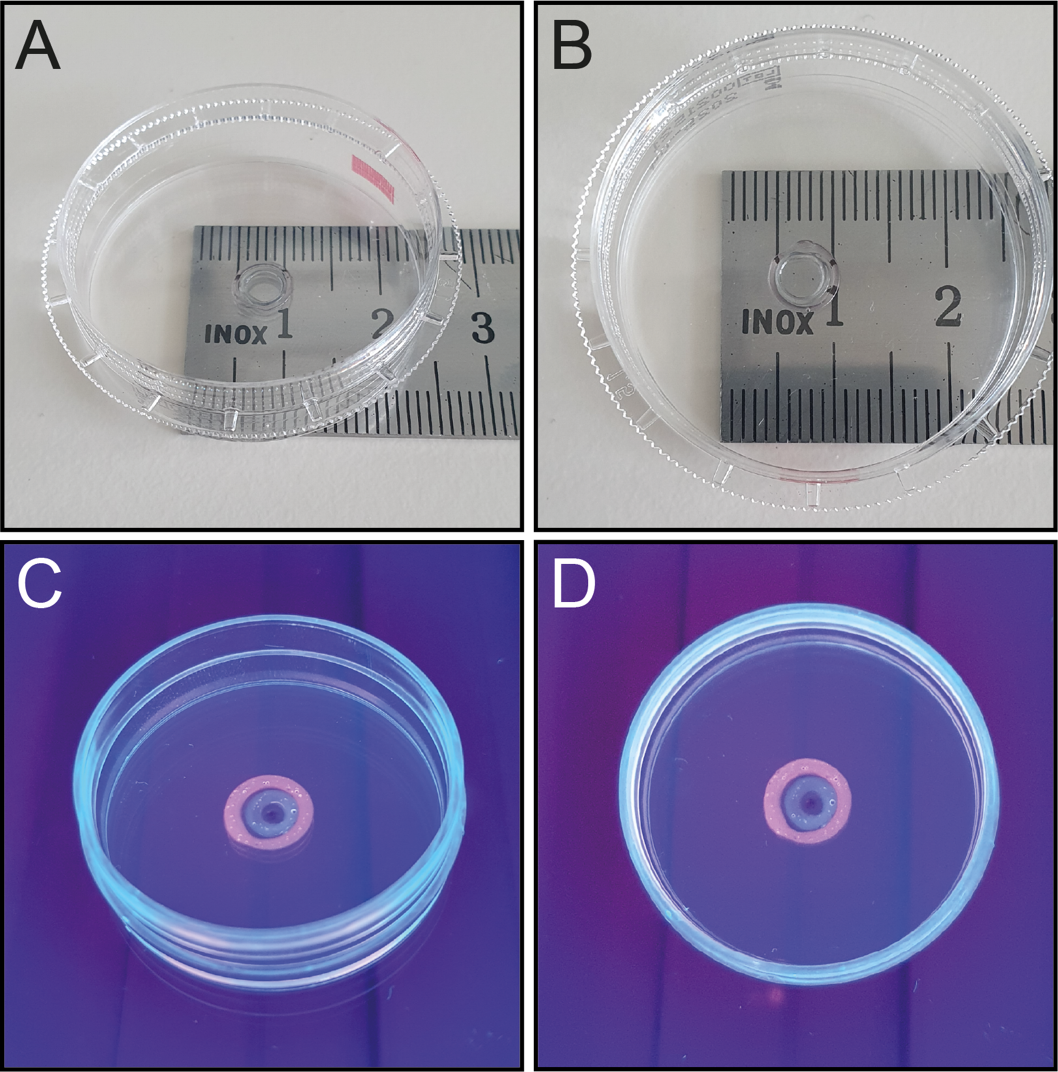
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**Figure S10:** IGF-I ELISA standard curve for Increlex® (IGF-I) and IGF-PSL-POx-b-POzi. Red dots were not measurable and set to maximal possible measurable value.

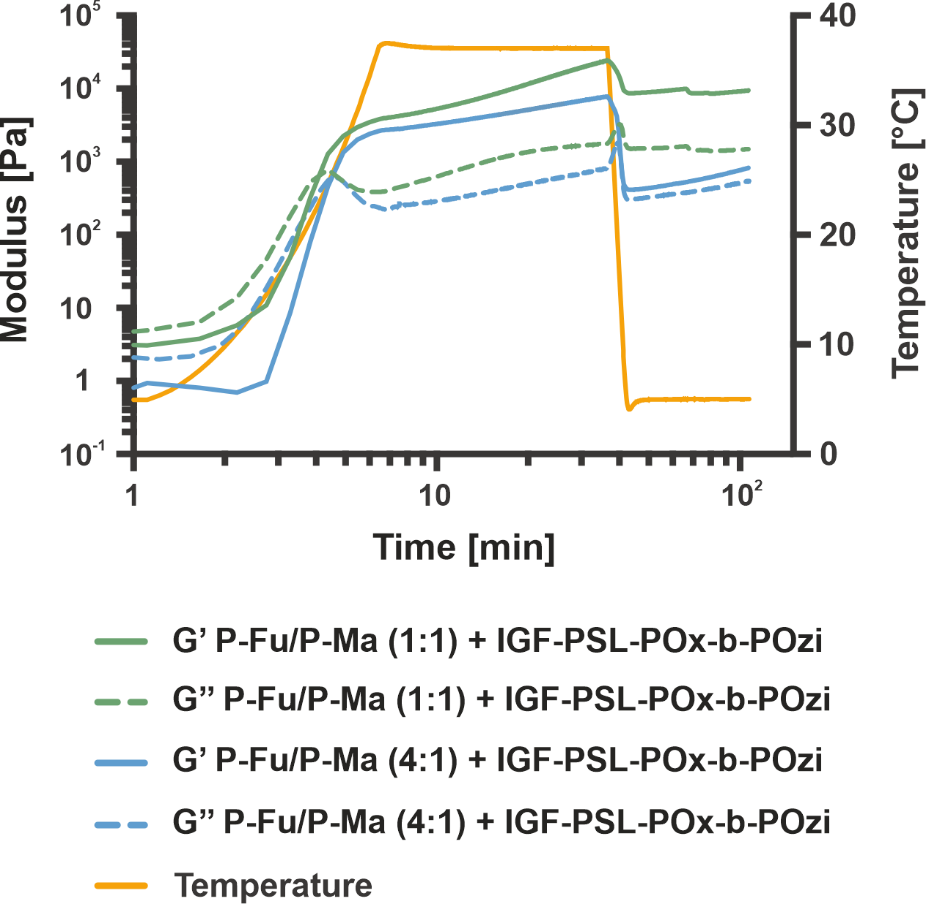
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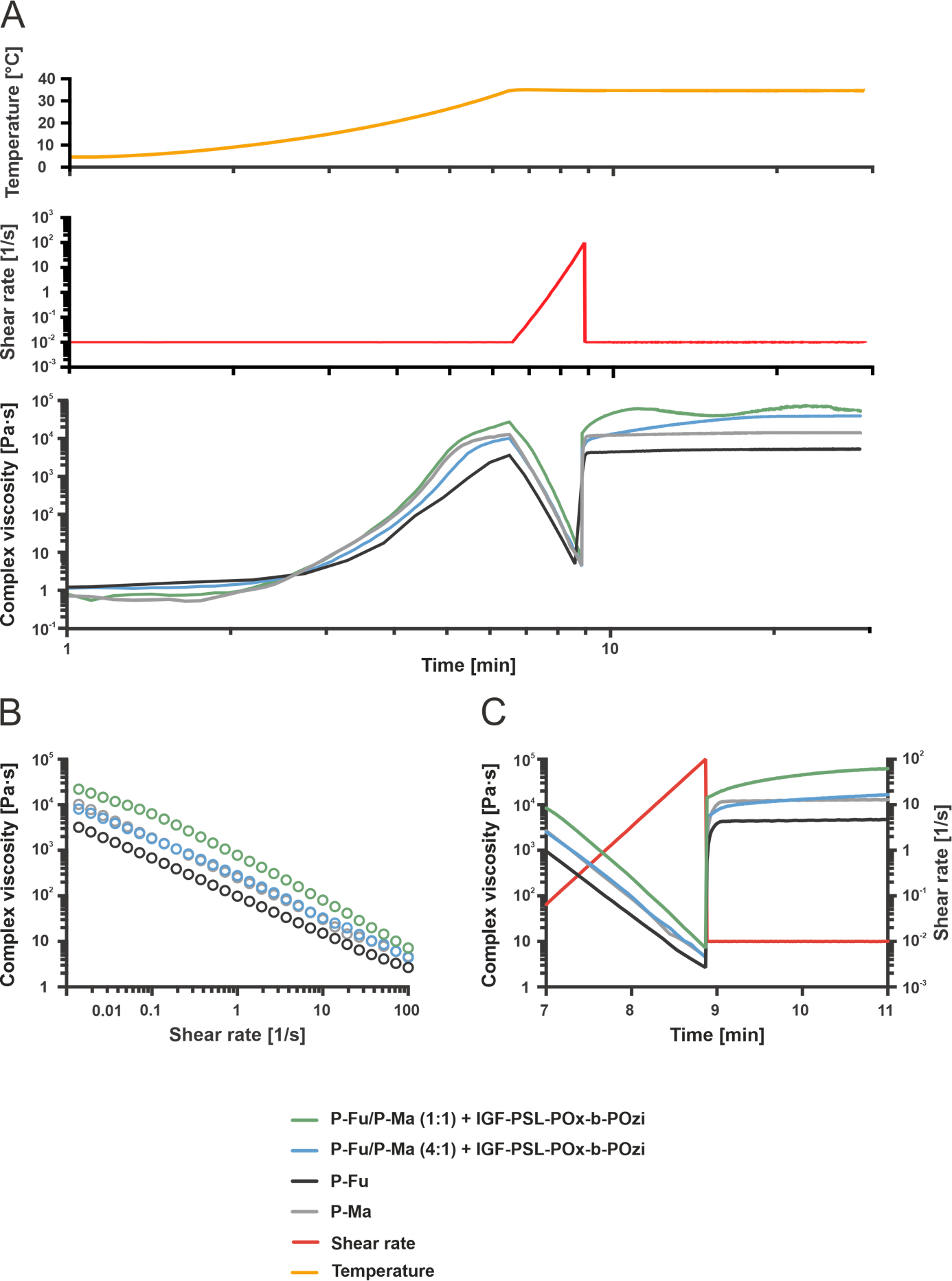
Figure S11: WST-1 standard curve of IGF-I (n=3).



**Figure S12:** (A-B) 3D printed construct with IGF-PSL-POx-b-POzi. (C-D) 3D printed construct with IGF-PSL-POx-b-POzi (green) visualized via NHS-labelling with NHS Alexa FluorTM 488 and Texas Red C2 MaleimideTM (red; P-Fu modified via Diels-Alder chemistry)



**Figure S13:** Chemical cross-linking of POx-b-POzi hydrogels with IGF-PSL-POx-b-POzi in the temperature range of 5 to 37 °C at 25 wt.% aqueous solutions.



**Figure S14:** Rheological measurements of POx-b-POzi hydrogels simulating a printing process with and without the addition of IGF-PSL-POx-b-POzi. (A) Overview over the whole process. (B) Shear thinning behaviour of the the POx-b-POzi hydrogels. (C) Recovery of POx-b-POzi hydrogels.

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**Video S1:** Dynamics of PSL-bound IGF-I. IGF-I is shown in ribbon presentation (colored according to the initial secondary structure), the PSL is shown in yellow stick presentation, and the isopeptide bond in red. The data corresponds to 1 µs simulation time from simulation run 1. Motions of the protein are smoothed over 5 time frames to improve visual impression.