

Supplementary Information

Polymerization of a Peptide-based Enzyme Substrate

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General methods

All reagents were purchased from commercial sources and used without further purification unless otherwise indicated below. CD_2Cl_2 , CH_2Cl_2 , CH_3OH , and CD_3OD used in polymerization reactions were dried over CaH_2 and 5 Ångstrom molecular sieves and were then degassed with 3 freeze-pump-thaw cycles. Sealed ampules of DMF-d_7 (Cambridge Isotopes) were used without modification. Modified 2nd Generation Grubbs' Ruthenium initiator, $(\text{IMesH}_2)(\text{C}_5\text{H}_5\text{N})_2(\text{Cl})_2\text{Ru}=\text{CHPh}$, was prepared as previously described.¹ Polymerizations were performed under dry dinitrogen atmospheres. Monomer **7** was prepared as described.² Norbornenyl-glycine was prepared as described.³ Polymer polydispersity and molecular weight were determined by size-exclusion chromatography (Phenomenex Phenogel 5u **10**, 1K-75K, 300 x 7.80 mm in series with a Phenomex Phenogel 5u **10**, 10K-1000K, 300 x 7.80 mm (0.05 M LiBr in DMF)) using a Shimadzu LC-10ATVP pump equipped with a multi-angle light scattering detector (DAWN-HELIOS, Wyatt Technology), a refractive index detector (Hitachi L-2490) and a UV-Vis detector (SPD-10AVP) normalized to a polystyrene standard. The dn/dc values used were 0.179 for PPAs **1** and **2** and 0.14 for PPA **3** (this reflects the contribution from the lower dn/dc value of the homopolymer of hydrophilic monomer **6**, which has been measured to be 0.1). The ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury Plus spectrometer. Chemical shifts (^1H) and (^{13}C) are reported in δ (ppm) relative to the residual solvent peak. Mass spectra were obtained at the UCSD Chemistry and Biochemistry Molecular Mass Spectrometry Facility. D_h was determined by DLS on a Nano-ZS90- (Malvern) or DynaPro NanoStar (Wyatt). TEM images were acquired on a carbon Forvar grid (Ted Pella, Inc.) with 1% uranyl acetate stain on a FEI Tecnai G2 Sphera at 200 kV.

Spherical Micelle Formation

Separate solutions of PPAs **1** – **3**, dissolved in DMF to a final concentration of ~ 1 mg/mL, were stirred with a magnetic stirbar. Then, an equivalent volume of water was added dropwise over approximately 10 minutes. The resulting mixture was dialyzed against water for a minimum of 8 hours x 3 using Pierce Snakesin dialysis tubing (MWCO = 10 kDa).

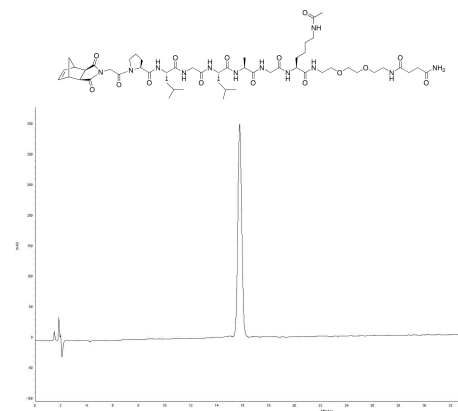
General Peptide Synthesis Procedures

Peptide monomers **4** and **5** were synthesized manually and peptide **8** was synthesized on an AAPPTec Focus XC automated synthesizer using standard Fmoc SPPS on Rink amide resin (H-Rink Amide-ChemMatrix[®], PCAS BioMatrix, Inc., Saint-Jean-sur-Richelieu, Quebec, Canada). Chain assembly was carried out with HATU or HBTU activation using a 5-fold excess of amino acid over the resin in DMF with DIEA as base. A stream of dry N_2 was used to agitate the reaction mixture. Fmoc removal was carried out with 20% piperidine in DMF (1 x 3 minutes, followed by 1 x 10 minutes). Cleavage of

peptides from the resin was achieved with 95% TFA, 2.5% triisopropylsilane (TIS), and 2.5% H_2O (cleavage cocktail). Crude peptide products were precipitated and washed with cold Et_2O and dissolved in 0.1% TFA in water (solvent A). A minimal amount of 0.1% TFA in acetonitrile (solvent B) was added to aid dissolution of any non-dissolved material. RP-HPLC analysis of peptides was performed on a Hitachi-Elite LaChrom L-2130 pump with a binary gradient. Detection was at 214 nm using an in-line UV-Vis detector (Hitachi-Elite LaChrom L-2420). For analysis, an analytical scale Phenomenex Jupiter 4u Proteo 90A column (150 x 4.60 mm) was utilized. For purification, a semi-preparative Phenomenex Jupiter 4u Proteo 90A column (250 x 10.0 mm) was utilized.

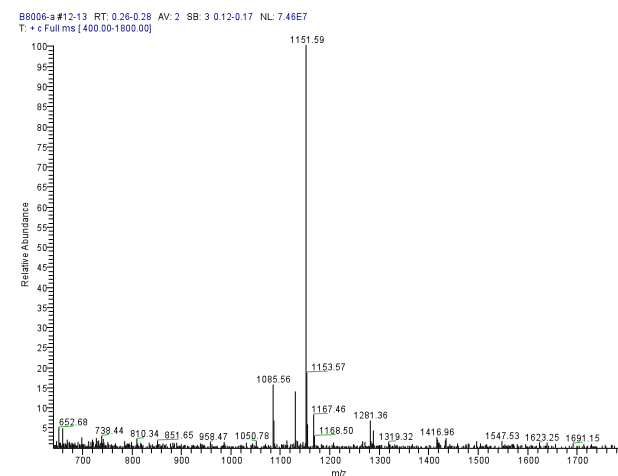
Identities and purities of the norbornenyl-peptide monomers were confirmed by ES-MS and RP-HPLC with detection at 214 nm:

Monomer 4:



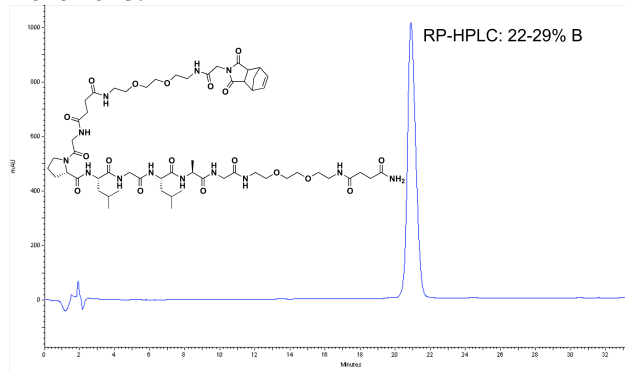
ES-MS:

found m/z 1129.43, expected 1129.62 $\text{M}+\text{H}^+$
found m/z 1151.59 expected 1151.61 $\text{M}+\text{Na}^+$



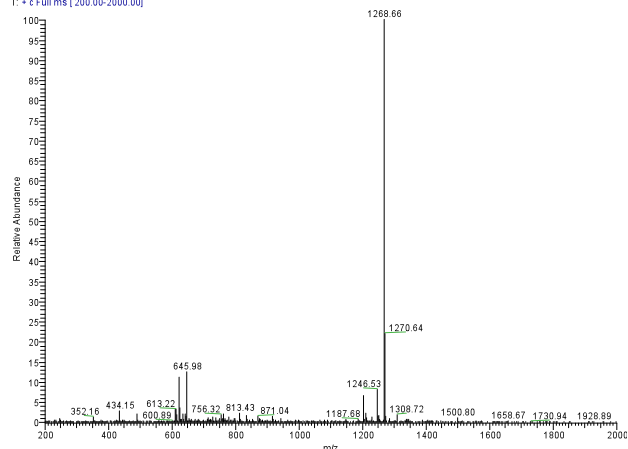
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Monomer 5:

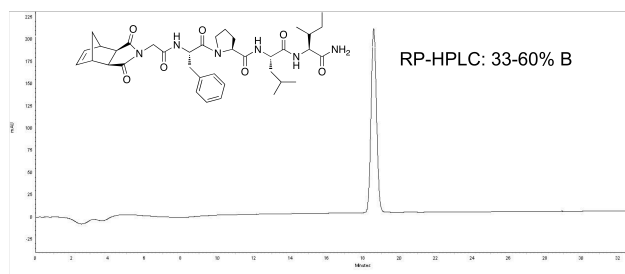


found m/z 1246.53, expected 1246.67 $M+H^+$
 found m/z 1268.66, expected 1268.65 $M+Na^+$
 found m/z 645.98, expected 645.82 $M+2Na^+$

LRC39-8-g #115-116 RT: 2.99-3.01 AV: 2 SB: 11 0.92-1.18 NL: 5.15E7
 T: + c Full ms [200.00-2000.00]

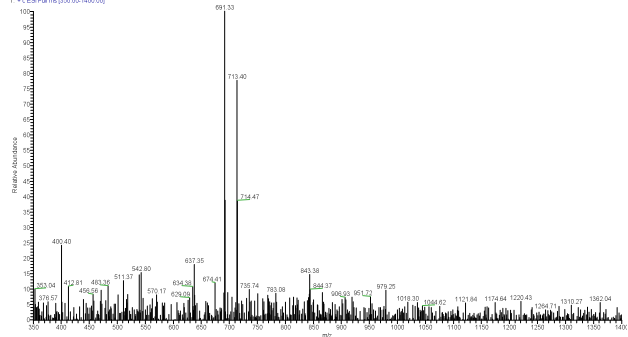


Monomer 8:



found m/z 691.33, expected 691.37 $M+H^+$
 found m/z 713.40, expected 713.36 $M+Na^+$

LAMA4-818-23 RT: 0.30-0.38 AV: 6 SB: 0 0.03-0.09 NL: 1.21E7
 T: + c Full ms [100.00-1400.00]



2-(2,5,8,11-tetraoxatridecan-13-yl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisindole-1,3(2H)-dione (6)

A solution of *cis*-5-Norbornene-*exo*-2,3-dicarboxylic anhydride (1.5g, 9.1 mmols) and 2,5,8,11-tetraoxatridecan-13-amine⁴ (2.27g, 11.0 mmoles) in toluene (50 mL) was heated at reflux overnight under a nitrogen atmosphere. The reaction mixture was cooled to room temperature, concentrated to dryness and purified by flash chromatography (2% MeOH in CH_2Cl_2) to give **6** as a light yellow oil, 3.12g (97%). ¹H NMR, 400MHz, $CDCl_3$, 1.35 (m, 1H), 1.47 (m, 1H), 2.66 (s, 2H), 3.24 (m, 2H), 3.36 (s, 3H), 3.5-3.7 (m, 16H), 6.26 (m, 2H). ¹³C NMR, 100MHz, $CDCl_3$, 37.64, 42.63, 45.19, 47.73, 58.95, 66.78, 69.77, 70.50, 71.83, 137.73, 177.91. HRMS Calc $M+Na$ = 376.1736, Obs. = 376.1730.

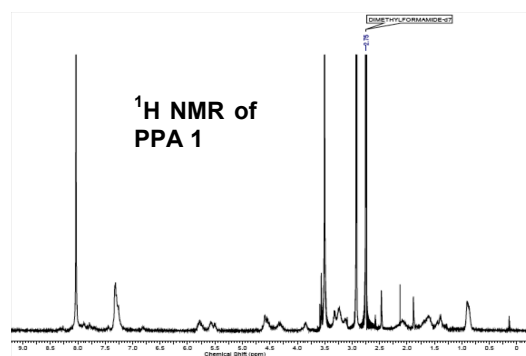
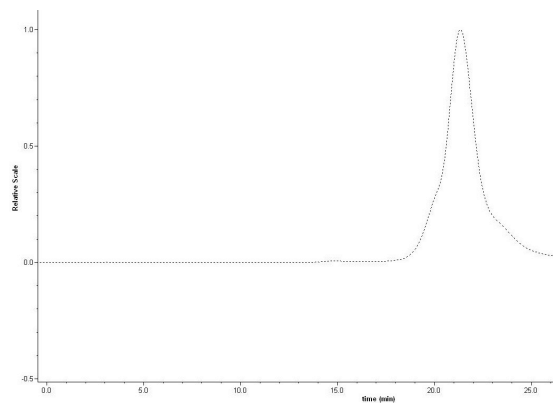
Polymerization procedures

PPA 1

To a stirred 0.4 mL solution containing **4** (3.95 mg, 3.5 μ mole) in a dry/degassed mixture of $CH_2Cl_2:CH_3OH$ (4:1) was added $(IMesH_2)(C_5H_5N)_2(Cl)_2Ru=CHPh$ (0.17 mg, 0.23 μ mole) pre-dissolved in 0.04 mL of the same solvent mixture. After 1 hour, 10% of the solution was removed and quenched with ethylvinyl ether. To the remaining solution was added **7** (2.92 mg, 11.54 μ mole) pre-dissolved in 0.1 mL of the same solvent mixture. After 1 hour, 0.1 mL of ethylvinyl ether was added to the reaction mixture, which was stirred for 20 minutes. The polymer was then precipitated with 12 mL of ice-cold diethyl ether and collected by centrifugation.

SEC-MALS (PPA 1):

Homopolymer of **4**: M_n = 15,240; M_w/M_n = 1.07; **DP** = 13.
 Copolymer of **4-b-7**: M_n = 44,630; M_w/M_n = 1.20; **DP** = 116.



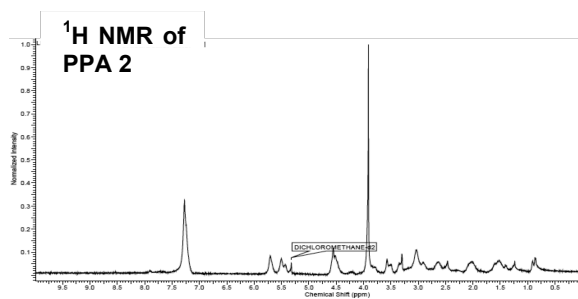
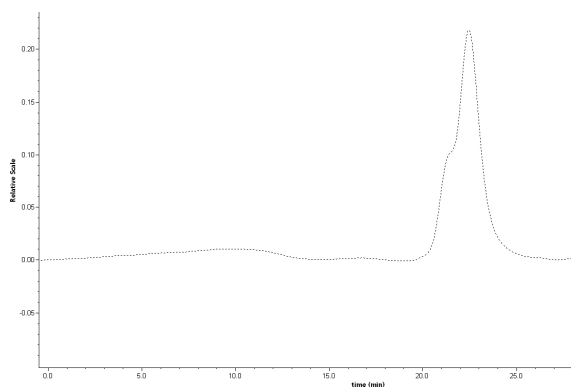
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PPA 2

To a stirred 0.4 mL solution containing the hydrophobic monomer **7** (5.3 mg, 22.5 μmole) in dry/degassed $\text{CD}_2\text{Cl}_2:\text{CD}_3\text{OD}$ (4:1) was added $(\text{IMesH}_2)(\text{C}_5\text{H}_5\text{N})_2(\text{Cl})_2\text{-Ru=CHPh}$ (0.218 mg, 0.3 μmole) pre-dissolved in 0.1 mL of the same solvent mixture. After 0.5 hour, 10% of the solution was removed and quenched with ethylvinyl ether and saved for later analysis. To the remaining solution was added norbornenyl-peptide monomer **5** (1.89 mg, 1.5 μmole) pre-dissolved in 0.15 mL of the same solvent mixture. After 1 hour, 0.1 mL of ethylvinyl ether was added to the reaction mixture, which was stirred for 20 minutes. The resulting solution was used directly for analysis and micelle formation without further purification.

SEC-MALS (PPA 2):

Homopolymer of **7**: $M_n = 17,010$; $M_w/M_n = 1.025$; $m = 74$.
Copolymer of **7-b-5**: $M_n = 23,570$; $M_w/M_n = 1.056$; $n = 5$.

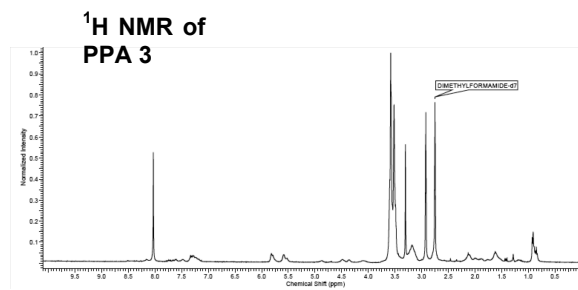
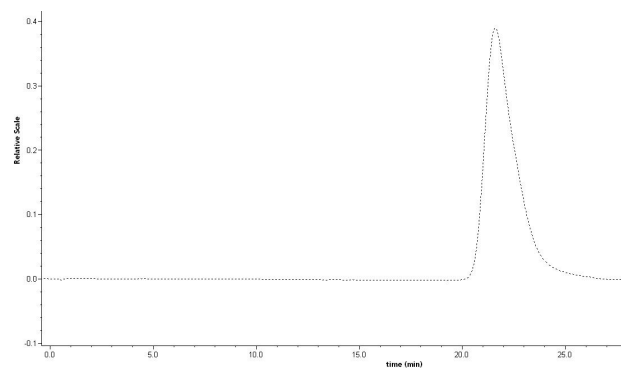


PPA 3

To a stirred 0.45 mL solution containing hydrophilic monomer **6** (5.2 mg, 14.7 μmole) in DMF-d_7 was added $(\text{IMesH}_2)(\text{C}_5\text{H}_5\text{N})_2(\text{Cl})_2\text{Ru=CHPh}$ (0.214 mg, 0.29 μmole) pre-dissolved in 0.05 mL of DMF-d_7 . After 1 hour, 20% of the solution was removed and quenched with ethylvinyl ether and saved for later analysis. To the remaining solution was added hydrophobic norbornenyl-peptide monomer **8** (3.25 mg, 11.8 μmole) pre-dissolved in 0.05 mL of the DMF-d_7 . After 7 hours, 0.02 mL of ethylvinyl ether was added to the reaction mixture. The resulting solution was used directly for analysis and micelle formation without further purification.

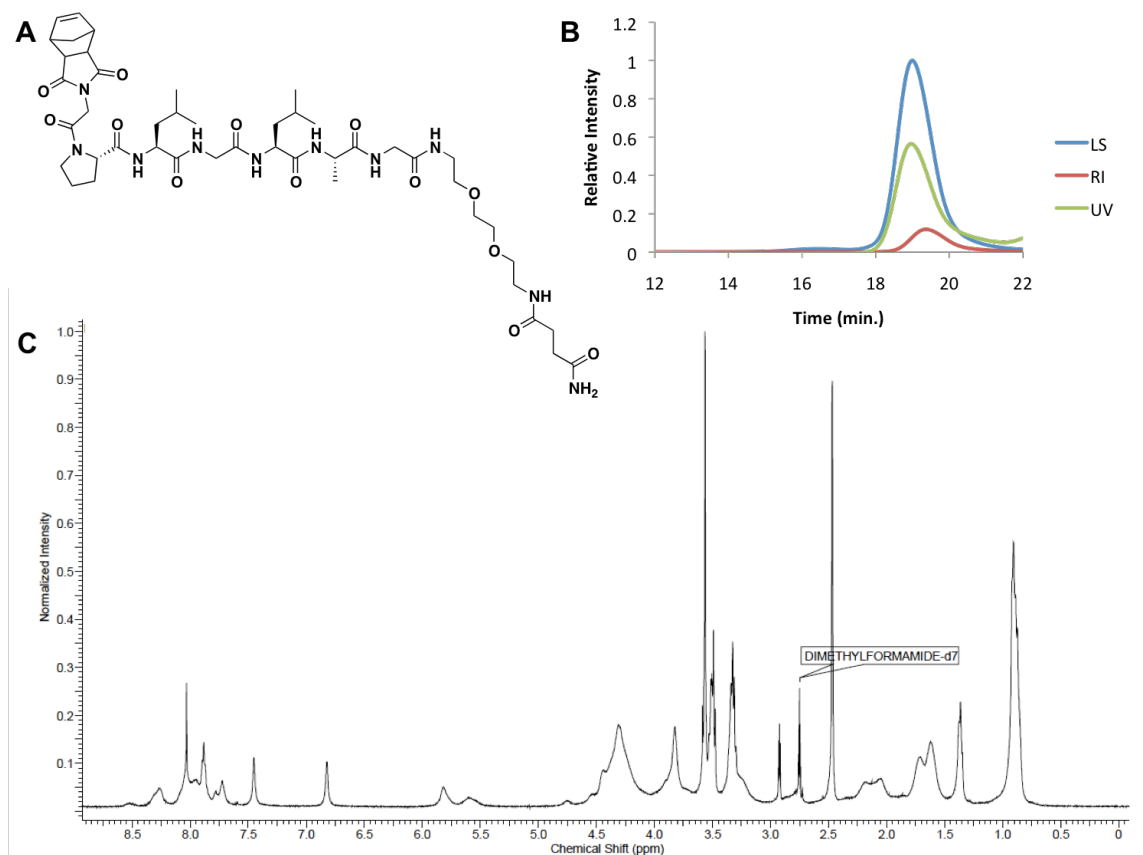
SEC-MALS (PPA 3):

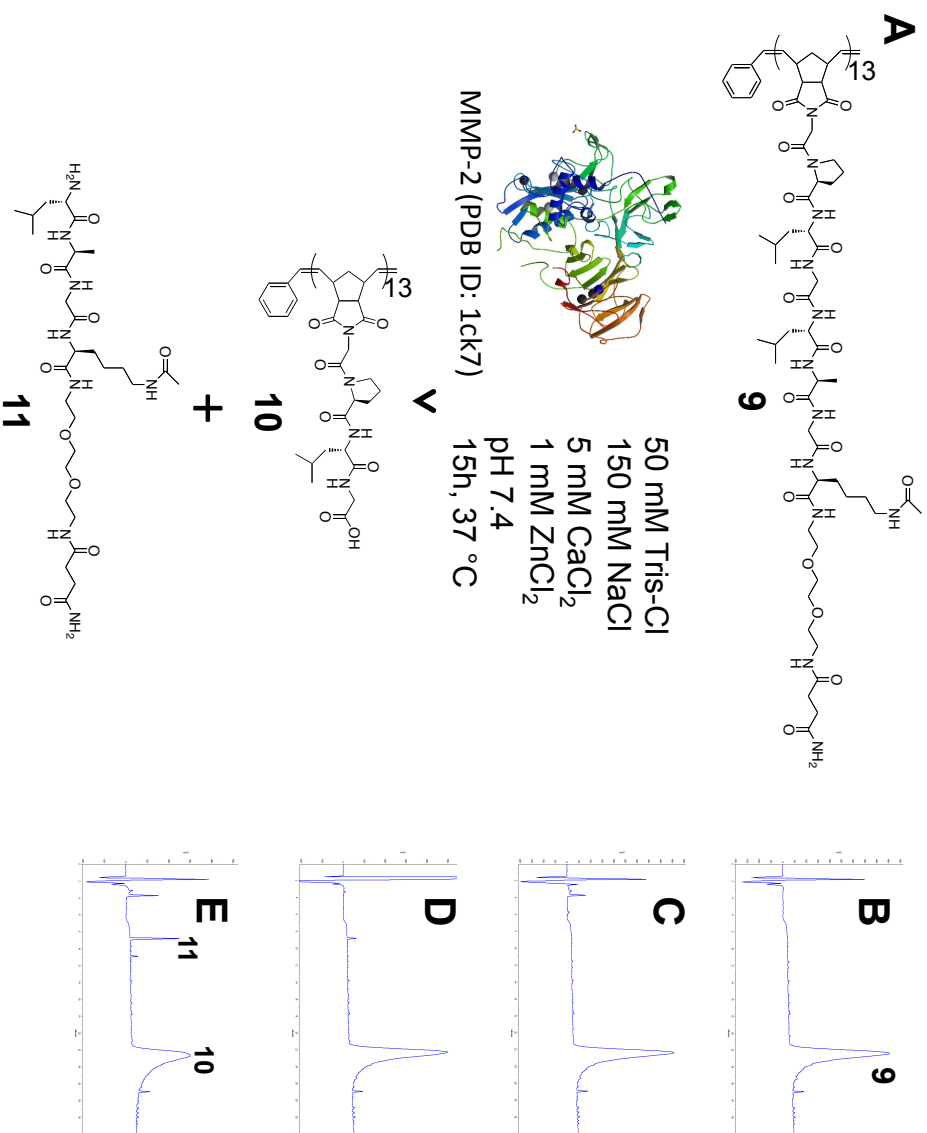
Homopolymer of **6**: $M_n = 20,820$; $M_w/M_n = 1.01$; $m = 59$.
Copolymer of **6-b-8**: $M_n = 28,130$; $M_w/M_n = 1.05$; $n = 11$.



SI Figure 1. Preparation of peptide-polymer with degree of polymerization = 131 discussed in the text.

To a stirred 0.43 mL solution containing hydrophilic monomer (18 mg, 18.8 μmole) in DMF-d7 was added $(\text{IMesH}_2)(\text{C}_5\text{H}_5\text{N})_2(\text{Cl})_2\text{Ru}=\text{CHPh}$ (0.136 mg, 0.188 μmole) pre-dissolved in 0.02 mL of DMF-d7. After 48 hours, the solution was quenched with 0.01 mL of ethylvinyl ether. The resulting solution was used directly for analysis and without further purification. SEC-MALS: $M_n = 125,300$, $M_w/M_n = 1.012$, $DP = 131$.





Conditions

Cleavage buffer:

50 mM Tris-Cl
150 mM NaCl
5 mM CaCl₂
1 mM ZnCl
pH 7.4

[**9**] = 0.1 mM

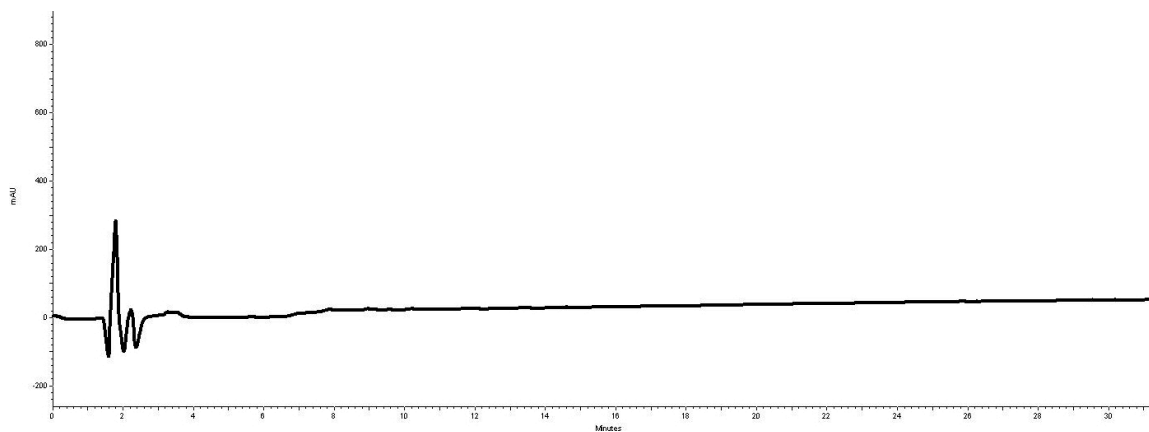
[MMP-2] = 10 nM.

Reactions run at 37 °C for 15 hours.

Human MMP-2 (matrix metalloproteinase 2) derived from Human Rheumatoid Synovial Fibroblast, Calbiochem) was pre-activated for 1h at 37 °C with 2.5 mM 4-aminophenylmercuric acetate.

SI Figure 2. Peptide polymer **9** retains the ability to function as an enzyme substrate.

- (A) When exposed to MMP-2, a proteinase that cleaves the -GL- motif in peptide polymer **9** ($M_n = 12,830$ g/mole; DP = 13; PDI = 1.001), the expected products are truncated peptide polymer **10**, and the free C-terminal peptide fragment **11**.
- RP-HPLC analysis of MMP-2 mediated cleavage of peptide polymer **9**:
- (B) Peptide polymer **9** without MMP-2.
- (C) Peptide polymer **9** treated with heat inactivated MMP-2.
- (D) Peptide polymer **9** treated with MMP-2 that was pre-treated with 35 mM EDTA (EDTA inhibits MMP-2 via chelation of catalytic divalent zinc).
- (E) Peptide polymer **9** treated with active MMP-2. The peak corresponding to peptide fragment **11** was analyzed by ES-MS and shown to correspond to the expected molar mass (found m/z 659.4, expected 659.40 $M+H^+$; found m/z 681.4, expected 681.39 $M+Na^+$).



SI Figure 2, continued. Peptide displayed on particle 2 (PPA 2) is not cleaved by MMP-2

(F) After treatment of PPA2 particles with MMP-2 under the same conditions described above for the soluble homopolymer of the same peptide (except that incubation time was extended to 48h), no proteolysis product was observed. Note lack of peak seen in SI Figure 2E at approximately 9 minutes. The concentration of PPA 2 used in this experiment reflects the same concentration as that used above for the soluble homopolymer with respect to peptide.

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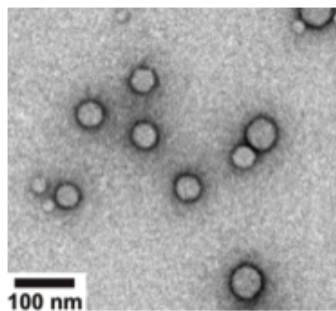
SI Figure 3. TEM of PPAs 2 and 3. Supplement to Figure 1.

(A) TEM of PPA 2

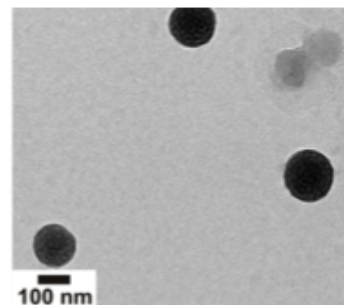
(B) TEM of PPA 3

(C) TEM of PPA 3 at lower magnification to demonstrate population of particles

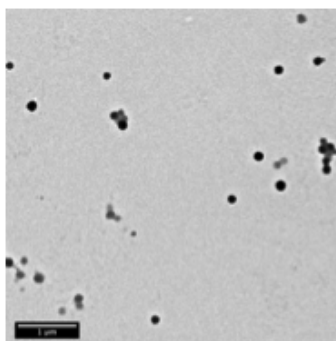
A



B



C



References

- (1) Sanford, M. S.; Love, J. A.; Grubbs, R. H. *Organometallics* 2001, 20, 5314-5318.
- (2) Chien, M. P.; Rush, A. M.; Thompson, M. P.; Gianneschi, N. C. *Angew Chem Int Ed Engl* 2010, 49, 5076-80.
- (3) Conrad, R. M.; Grubbs, R. H. *Angew Chem Int Ed Engl* 2009, 48, 8328-30.
- (4) Infante, M. R.; Seguer, J.; Pinazo, A.; Vinardell, M. P. *Journal of Dispersion Science and Technology* 1999, 20, 621-642.