Supporting Information

Polymer endgroup control through a decarboxylative cobalt-mediated radical polymerization: new avenues for synthesising peptide, protein, and nanomaterial conjugates

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I. General experimental

Starting materials and reagents were purchased from Sigma-Aldrich or Oakwood and were used as supplied. Dichloromethane (CH$_2$Cl$_2$) were dried by passing over activated alumina. DMF was dried over 4 Å molecular sieves and distilled under reduced pressure, and stored over 4 Å molecular sieves. Unless otherwise stated, all reactions were conducted in flame-dried glassware under an atmosphere of nitrogen. Methyl acrylate was dried over MgSO$_4$ and distilled under reduced pressure. Dimethyl acrylamide was dried over MgSO$_4$ and passed through neutral alumina to remove inhibitor.

Proton (1H) and carbon (13C) NMR spectra were recorded on a Bruker DRX600 spectrometer operating 600 MHz for proton and at 150 MHz for carbon nuclei, a Bruker DRX400 spectrometer operating at 400 MHz for proton and 100 MHz for carbon nuclei, and a Bruker DRX300 spectrometer operating at 300 MHz for proton nuclei. 2D correlation spectra were recorded on a Bruker DRX400 spectrometer. Infrared spectra ($\nu_{\text{max}}$) were recorded on an Agilent Cary 630 FTIR Spectrometer. High resolution mass spectra (HRMS) (ESI) were recorded on a Bruker BioApex 47e FTMS fitted with an Analytical electrospray source using NaI for accurate mass calibration. MALDI-TOF MS measurements were performed on a Bruker UltraflexXtreme MALDI-TOF/TOF mass spectrometer in reflectron positive mode using a 40 mg/mL DCTB (trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile) matrix in THF, with 1 mg/mL Ag(CF$_3$COO). The laser was operated at 200 Hz with 2.0% baseline offset, low real-time smoothing and a reflectron detector gain of 2457V. Number-average molecular weight (Mn) and dispersity (Mw/Mn) of the resulting polymers were determined using an EcoSEC TOSOH gel permeation chromatography (GPC), calibrated against polystyrene standards. Samples were run using TOSOH alpha 4000 and 2000 columns and the instrument was equipped with both a refractive-index (IR) and ultraviolet (UV) detectors (UV detection, l $= 280$ nm). DMF (with 10 mM LiBr) was used as mobile phase with a flow rate of 1.0 mL/min.

The scanning electron microscopy (SEM): The SEM images of all samples were obtained on a Magellan 400 FESEM (FEI, USA) operating at an accelerating voltage of 5 kV, 2.0 spot size. All mounted samples were sputter coated with iridium.

Sample preparation:

5 mg of pristine graphene or graphene-PMA powder was dispersed in 20 mL ethanol solvent to yield graphene or graphene-PMA/EtOH suspension, following by an ultra-sonification process for 1 hour. Then, 5 mL graphene or graphene-PMA/EtOH supernatant was filtered on commercial 13 mm porous aluminium oxide substrates. The resulting layers with substrates were dried at room temperature (20 °C) over night. After that, they were mounted on the SEM sample holders and coated with iridium.
II. Synthesis of activated esters

Scheme S1 - N-Cbz-phe-NHPI ester 2

\[
\begin{align*}
\text{N-Cbz-phenylalanine} (500 \text{ mg, 1.67 mmol}), \text{N-Hydroxyphthalimide} (\text{NHPI, 163 mg, 1.67 mmol}), \\
\text{and } \text{N, N-dicyclohexylcarbodiimide} (\text{DCC, 413 mg, 2 mmol}), \text{and } 4\text{-dimethylamino pyridine} (\text{DMAP, 10.2 mg, 0.08 mmol}) \text{ were added to a 100-mL round bottom flask with a stir bar,} \\
\text{followed by dichloromethane (20 mL). The solution was stirred at room temperature overnight} \\
\text{under nitrogen. Diethyl ether (50 mL) was added, and the reaction mixture was filtered using} \\
\text{celite. The filtrate was concentrated under reduced pressure, and purified by silica gel} \\
\text{chromatography, eluted in dichloromethane to give ester 2 (74.50 mg, 10 \%).} \\
^1\text{H-NMR} (400 \text{ MHz, DMSO-}d_6) \delta 8.03 - 7.90 (m, 4H), \delta 7.43 - 7.21 (m, 10H), \delta 5.03 (s, 2H), 4.82 \\
(\text{td, } J = 9.6, 4.6 \text{ Hz, 1H}), \delta 3.32 (\text{dd, } J = 13.9, 4.6 \text{ Hz, 1H}), \delta 3.11 (\text{dd, } J = 13.8, 10.4 \text{ Hz, 1H}). \\
^{13}\text{C-NMR} (100 \text{ MHz, DMSO-}d_6) \delta 169.2, 161.7, 156.0, 136.7, 136.4, 135.6, 129.4, 128.4, 128.2, 127.9, \\
127.6, 127.3, 126.9, 124.1, 65.8, 53.9, 36.4.
\end{align*}
\]

Scheme S2 - N-Cbz-phe-TCNHPI ester 3

\[
\begin{align*}
\text{N-Cbz-phenylalanine} (1.496 \text{ g, 5 mmol}), \text{N-Hydroxytetrachlorophthalimide} (\text{TCNHPI, 1.50 mg,} \\
\text{5 mmol}), \text{and } \text{N, N-dicyclohexylcarbodiimide} (\text{DCC, 1.03 mg, 5 mmol}) \text{ were added to a 250-mL} \\
\text{round bottom flask with a stir bar, followed ethyl acetate (75 mL). The solution was stirred at room} \\
\text{temperature for 3 h under nitrogen. Then the reaction mixture was filtered through celite.} \\
\text{The filtrate was concentrated under reduced pressure, and purified by silica gel} \\
\text{chromatography, eluted in dichloromethane to give N-Cbz-phe-TCNHPI ester 3 as a white solid} \\
(1.25 \text{ g, 43 \%).} \\
^1\text{H-NMR} (400 \text{ MHz, Acetone-}d_6) \delta 7.48 - 7.40 (m, 2H), \delta 7.38 - 7.23 (m, 8H), \delta 5.06 (s, 2H), \delta \\
4.99 (\text{ddd, } J = 10.0, 8.6, 4.9 \text{ Hz, 1H}), \delta 3.46 (\text{dd, } J = 14.1, 4.9 \text{ Hz, 1H}), \delta 3.23 (\text{dd, } J = 14.1, 10.0 \text{ Hz,} \\
1H). \text{ }^1\text{H-NMR} (600 \text{ MHz, DMSO-}d_6) \delta 7.40 - 7.20 (m, 10H), \delta 5.07 - 4.99 (m, 2H), \delta 4.79 (\text{ddd, } J
= 10.5, 8.2, 4.8 Hz, 1H), δ 3.27 (dd, J = 13.9, 4.9 Hz, 1H), δ 3.09 (dd, J = 13.8, 10.5 Hz, 1H). $^{13}$C-NMR (150 MHz, DMSO-$d_6$) δ 168.6, 157.4, 155.8, 138.3, 136.6, 136.2, 129.3, 129.0, 128.4, 128.3, 127.8, 127.6, 126.8, 125.3, 65.7, 54.9, 53.7. HRMS-ESI (m/z): [M+Na]$^+$ calcd for C$_{25}$H$_{16}$Cl$_4$N$_2$NaO$_6$+$^+$, 602.9655; found 602.9615.

**Scheme S3 - Boc-D-glu-OBzl-TCNHPI ester S6**

Boc-D-Glu-OBzl (1686 mg, 5 mmol), N-Hydroxyltetrachlorophthalimide (TCNHPI, 1504 mg, 5 mmol), and N, N-dicyclohexylcarbodiimide (DCC, 1032 mg, 5 mmol) were added to a 250-mL round bottom flask with a stir bar, followed by adding 75 mL of ethyl acetate. The reaction was conducted at room temperature for 3 h under nitrogen atmosphere. Then, the crude was filtered using celite to remove dicyclohexyl urea (DCU). The filtrate was concentrated using rotary evaporator, and purified by silica gel chromatography, eluted in dichloromethane to give Boc-D-glu-OBzl-TCNHPI S6 ester, as a white solid (140 mg, 4.5%).

$^1$H-NMR (400 MHz, Acetone-$d_6$) δ 7.47 – 7.30 (m, 5H), δ 5.28 – 5.13 (m, 2H), δ 4.38 (d, J = 5.6 Hz, 1H), δ 2.95 (tdd, J = 15.5, 9.3, 6.4 Hz, 2H), δ 2.34 (q, J = 8.5, 7.4 Hz, 1H), δ 2.26 – 2.13 (m, 1H), δ 1.41 (s, 9H). $^{13}$C-NMR (150 MHz, dmso-$d_6$) δ 169.0, 159.9, 157.6, 155.6, 139.3, 137.9, 135.9, 128.4, 127.7, 125.2, 78.5, 66.1, 54.9, 52.5, 28.1, 26.9. HRMS-ESI (m/z): [M-H] $^+$ calcd for C$_{25}$H$_{21}$Cl$_4$N$_2$O$_8$ $^+$, 617.0058; found 617.0084.

**Scheme S4 - Biotin-TCNHPI ester S7**

CITU (736.2 mg, 1.35 mmol) was dissolved by dried DMF (9 mL) in a round bottom flask equipped with a stir bar under nitrogen flow. Then, Biotin (300 mg, 1.23 mmol) was added into the CITU solution, followed by N-methylmorpholine (NMM, 273.3 mg, 2.70 mmol). The reaction was stirred at room temperature for 2 h under nitrogen atmosphere. Then, a large amount of water was added into the crude reaction. The solid formed was filtered to get the product S7.
(200 mg, 31%). The product was dried using high vacuum and used without further purification.

**1H-NMR** (600 MHz, DMSO-$d_6$) δ 6.44 (s, NH), δ 6.37 (s, NH), δ 4.31 (ddt, $J = 7.6, 5.2, 1.1$ Hz, 1H), δ 4.16 (dd, $J = 7.8, 4.4, 1.8$ Hz, 1H), δ 3.13 (dd, $J = 8.3, 6.3, 4.4$ Hz, 1H), δ 2.84 (dd, $J = 12.4, 5.1$ Hz, 1H), δ 2.82 – 2.75 (m, 2H), δ 2.59 (d, $J = 11.8$ Hz, 1H), δ 1.74 – 1.41 (m, 6H).

**13C-NMR** (100 MHz, DMSO-$d_6$) δ 169.6, 162.7, 157.7, 139.3, 129.0, 125.2, 61.1, 59.2, 55.2, 29.9, 27.8, 27.5, 24.3.

**HRMS-ESI** (m/z): [M+H]$^+$ calc'd for $C_{18}H_{16}Cl_4N_3O_5S$, 525.9559; found 525.9584.

**Scheme S5 - Isosteviol-TCNHPI ester S12**

CITU (376.5 mg, 0.69 mmol, 1.1 equiv) was dissolved by dried DMF (5 mL) in a round bottom flask equipped with a stir bar under nitrogen flow. Then, isosteviol (200.0 mg, 0.63 mmol, 1 equiv) was added into the CITU solution, followed by N-methylmorpholine (NMM, 139.8 mg, 1.38 mmol, 2.2 equiv). The reaction was stirred at room temperature for 2 h under nitrogen atmosphere. Then, a large amount of water was added into the crude reaction. The solid formed was filtered and dried using high vacuum (253 mg, 67%). The ester was then used without further purification.

**1H-NMR** (400 MHz, CDCl$_3$) δ 2.73 (dd, $J = 18.6, 3.7$ Hz, 1H), δ 2.40 – 2.32 (m, 1H), δ 1.97 – 1.89 (m, 2H), δ 1.89 – 1.74 (m, 4H), δ 1.72 – 1.65 (m, 2H), δ 1.59 – 1.52 (m, 2H), δ 1.47 (s, 3H), δ 1.45 – 1.39 (m, 2H), δ 1.33 – 1.14 (m, 6H), δ 0.98 (s, 3H), δ 0.91 (s, 3H). **13C-NMR** (100 MHz, CDCl$_3$ δ 77.16) δ 222.2, 173.0, 157.9, 141.0, 130.5, 124.9, 57.3, 54.8, 54.4, 48.8, 48.5, 44.3, 41.4, 39.6, 39.5, 38.3, 38.1, 37.4, 29.1, 21.7, 20.5, 19.9, 19.0, 14.2. **HRMS-ESI** (m/z): [M-H+H$_2$O]$^+$ calc'd for $C_{28}H_{30}Cl_4NO_6$, 616.0833; found 616.0852.

**Scheme S6 3-N-Boc-4-(2,4,5-trifluorophenyl)butanoic-TCNHPI ester 18**
3-N-Boc-4-(2,4,5-trifluorophenyl)butanoic acid (1666 mg, 5 mmol), N-Hydroxytetrachlorophthalimide (TCNHPI, 1504 mg, 5 mmol), and N,N-dicyclohexylcarbodiimide (DCC, 1032 mg, 5 mmol) were added to a 250-mL round bottom flask with a stir bar, followed by adding 75 mL of ethyl acetate. The reaction was conducted at room temperature for 2 h under nitrogen atmosphere. Then, the crude was filtered using celite to remove dicyclohexyl urea (DCU). The filtrate was concentrated using rotary evaporator, and purified by silica gel chromatography, eluted in dichloromethane to give 3-N-Boc-4-(2,4,5-trifluorophenyl)butanoic-TCNHPI ester, as a white solid (178 mg, 5.7%).

**1H-NMR** (600 MHz, DMSO-\(d_6\)) \(\delta\) 7.57 – 7.25 (m, 2H), 6.97 (d, \(J = 9.1\) Hz, 1H), 4.13 (qt, \(J = 9.5, 4.9\) Hz, 1H), 3.17 – 2.81 (m, 3H), 2.70 – 2.55 (m, 1H), 1.27 (s, 9H).

**13C NMR** (151 MHz, DMSO) \(\delta\) 167.21, 157.54, 156.07 (dd, \(J = 243.1, 9.7\) Hz), 154.73, 147.86 (dt, \(J = 245.9, 13.4\) Hz), 146.67 – 144.52 (m), 139.32, 128.98, 125.24, 122.63 (dd, \(J = 18.7, 5.0\) Hz), 119.37 (dd, \(J = 19.0, 6.4\) Hz), 105.41 (dd, \(J = 29.3, 21.0\) Hz), 77.89, 47.13, 35.64, 33.02, 27.99. **HRMS-ESI** (m/z): [M-Boc+2H]+ or [C\(_{18}\)H\(_{19}\)Cl\(_4\)F\(_3\)N\(_2\)O\(_4\)+H]+ calcd for C\(_{23}\)H\(_{27}\)Cl\(_4\)F\(_3\)N\(_2\)O\(_6\), 516.9312; found 516.9283.

**Scheme S7 - Pentafluorophenyl glutarate-TCNHPI ester 20**

Pentafluorophenol (170 mg, 0.92 mmol, 1.1 equiv) and glutaric anhydride (95 mg, 0.84 mmol, 1 equiv) were dissolved by dried DMF (3 mL) in a round bottom flask equipped with a stir bar under nitrogen flow. After 3 h stirring at room temperature under nitrogen atmosphere, CITU and NMM were added to the reaction mixture. The reaction was stopped by adding a large amount of water after DCU was formed and no liquid left in the reaction mixture (30 minutes). The solid formed was filtered and dried using high vacuum (287 mg), then purified by silica gel chromatography, eluted in dichloromethane to give pentafluorophenyl glutarate-TCNHPI ester 20, as a white solid (97 mg, 20%).

**1H NMR** (600 MHz, Benzene-\(d_6\)) \(\delta\) 2.10 (t, \(J = 7.4\) Hz, 1H), 2.05 (t, \(J = 7.3\) Hz, 1H), 1.60 (p, \(J = 7.4\) Hz, 1H). **13C NMR** (151 MHz, Benzene-\(d_6\)) \(\delta\) 168.84, 168.32, 157.48, 141.31 (142.16 m and 140.47 m, \(J = 255.2\) Hz), 140.32, 139.61 (140.56 m and 138.67 m, \(J = 285.4\) Hz), 138.05 (138.89 m and 137.21 m, \(J = 253.7\) Hz), 130.09, 125.22 m, 124.85, 31.41, 29.40, 19.59.
III – Fmoc-solid-phase peptide synthesis (SPPS)

Peptide substrates for the synthesis of functionalised polymers 13, 14, and 15 were prepared using Fmoc-SPPS according to the general protocols below. Peptides S13 and S14 were prepared on 2-chlorotrityl chloride resin and peptide S15 was prepared on Rink amide resin.

Scheme S8. A) Synthesis of peptides S13 and S14 on 2-chlorotrityl chloride resin; B) Synthesis of peptide S15 on Rink amide resin

Loading 2-chlorotrityl chloride resin

2-Chlorotrityl chloride resin (1.0 equiv., substitution = 1.4 mmol/g) was swollen in DCM for 30 min then washed with DCM (5 × 3 mL) and DMF (5 × 3 mL). A solution of the Fmoc-Leu-OH or Fmoc-Phe-OH (4.0 equiv.) and N,N-diisopropylethylamine (DIEA, 8.0 equiv.) in DMF (final concentration 0.1 M with respect to the resin) was added to the resin (1.0 equiv.) and agitated at room temperature. After 16 h, the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL), and DMF (5 × 3 mL).

Resin Capping: A solution of DCM/MeOH/DIEA (17:2:1 v/v/v) was added to the resin. After 15 min the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL). The resin-bound residue was submitted to iterative peptide assembly (Fmoc-SPPS).

Loading Rink amide resin

Rink amide resin (1.0 equiv., substitution = 0.8 mmol/g) was swollen in dry DCM for 5 min then washed with DCM (5 × 3 mL) and DMF (5 × 3 mL). To remove the Fmoc group, the resin was treated with piperidine/DMF (1:9 v/v, 2 × 3 min) and washed with DMF
(5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL). A solution of Fmoc-Phe-OH (4.0 equiv.), PyBOP (4.0 equiv.) and N-methylmorpholine (NMM, 8.0 equiv.) in DMF (final concentration 0.1 M with respect to the resin) was added to the resin (1.0 equiv.) and agitated at room temperature. After 2-3 h, the resin was washed with DMF (5 x 3 mL), DCM (5 x 3 mL), and DMF (5 x 3 mL). A capping step was performed as described in the general protocols below and the resin-bound material was submitted to iterative peptide assembly (Fmoc-SPPS).

**General iterative peptide assembly (Fmoc-SPPS)**

Peptides were elongated using iterative Fmoc-solid-phase peptide synthesis (Fmoc-SPPS), according to the following general protocols:

*Deprotection:* The resin was treated with piperidine/DMF (1:9 v/v, 2 × 3 min) and washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL).

*General amino acid coupling:* A preactivated solution of protected amino acid (4 equiv.), PyBOP, and N-methylmorpholine (NMM) (8 equiv.) in DMF (final concentration 0.1 M with respect to the resin) was added to the resin. After 1 h, the resin was washed with DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL).

*Capping:* A solution of acetic anhydride/pyridine (1:9 v/v) was added to the resin. After 3 min the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL).

*Cleavage:* A mixture of TFA/iPr3SiH/water (90:5:5 v/v/v, 3 mL) was added to the resin. After 2 h, the resin was washed with TFA (3 x 2 mL) and DCM (3 x 2 mL).

*Work-up:* The combined cleavage solution and TFA and DCM washes were concentrated under a stream of inert gas (N2). The residue was treated with cold Et2O to precipitate the peptide, which was used without further purification.
**Peptide S13**

Peptide S13 was prepared on 2-chlorotrityl chloride resin. Fmoc-Phe-OH was loaded onto the resin followed by elongation with Fmoc-Gly-OH and Cbz-Ala-OH according to the general methods.

**\(^1\)H NMR** (400 MHz, Methanol-\(d_4\)): \(\delta\) 7.45 – 7.11 (m, 10H), 5.13 – 5.03 (m, 2H), 4.76 – 4.59 (m, 1H), 4.12 (q, \(J = 7.1\) Hz, 1H), 3.94 (d, \(J = 16.9\) Hz, 1H), 3.74 (d, \(J = 16.9\) Hz, 1H), 3.20 (dd, \(J = 13.9, 5.1\) Hz, 1H), 3.02 (dd, \(J = 13.7, 8.4\) Hz, 1H), 1.35 (d, \(J = 7.2\) Hz, 3H); **\(^{13}\)C NMR** (101 MHz, Methanol-\(d_4\)): \(\delta\) 176.0, 174.4, 171.3, 158.4, 138.4, 138.1, 130.4, 129.5, 129.0, 128.9, 127.8, 67.8, 55.2, 52.3, 43.3, 38.4, 17.8. **Note:** One aromatic carbon signal is overlapping; **LRMS (ESI\(^{+}\))**: Calcd mass [M+H]\(^{+}\): 428.18; mass found: 428.15 [M+H]\(^{+}\)

**Peptide S14**

Peptide S14 was prepared on 2-chlorotrityl chloride resin. Fmoc-Leu-OH was loaded onto the resin followed by elongation with Fmoc-Pro-OH, Fmoc-Ala-OH, Fmoc-Gly-OH, and Fmoc-Tyr(\(^t\)Bu)-OH. Before cleavage, the resin was deprotected and capped according to the general methods.

**\(^1\)H NMR** (400 MHz, Methanol-\(d_4\)): \(\delta\) 7.05 (d, \(J = 7.9\) Hz, 2H), 6.70 (d, \(J = 7.9\) Hz, 2H), 4.59 (q, \(J = 7.0\) Hz, 1H), 4.52 – 4.32 (m, 3H), 3.91 (d, \(J = 16.8\) Hz, 1H), 3.84 – 3.74 (m, 1H), 3.70 (d, \(J = 17.1\) Hz, 1H), 3.66 – 3.59 (m, 1H), 3.04 (dd, \(J = 13.9, 6.4\) Hz, 1H), 2.82 (dd, \(J = 13.8, 8.7\) Hz, 1H), 2.27 – 2.13 (m, 1H), 2.15 – 1.96 (m, 3H), 1.92 (s, 3H), 1.87 – 1.72 (m, 1H), 1.63 (t, \(J = 7.3\) Hz, 2H), 1.37 (d, \(J = 6.9\) Hz, 3H), 0.94 (dd, \(J = 15.3, 6.4\) Hz, 6H); **\(^{13}\)C NMR** (101 MHz, Methanol-\(d_4\)): \(\delta\) 175.9, 174.5, 174.4, 173.3, 173.3, 171.1, 157.3, 131.2, 129.1, 116.2, 61.2, 57.1, 52.1, 43.3, 41.6, 37.7, 30.3, 25.9, 23.4, 22.4, 21.9, 16.8. **Note:** Two carbon signals are overlapping/obscured; **LRMS (ESI\(^{+}\))**: Calcd mass [M+H]\(^{+}\): 562.29; mass found: 562.26 [M+H]\(^{+}\)
Peptide S15 was prepared on Rink amide resin. Fmoc-Phe-OH was loaded onto the resin followed by elongation with Fmoc-Glu(tBu)-OH, and Fmoc-Ala-OH. Before cleavage, the resin was deprotected and capped according to the general methods.

$^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.37 – 7.12 (m, 5H), 4.68 – 4.56 (m, 1H), 4.32 – 4.15 (m, 2H), 3.29 – 3.20 (m, 1H), 3.07 – 2.89 (m, 1H), 2.33 – 2.21 (m, 1H), 2.20 – 2.05 (m, 1H), 2.07 – 1.97 (m, 3H), 1.99 – 1.92 (m, 1H), 1.92 – 1.81 (m, 1H), 1.39 – 1.26 (m, 3H); $^{13}$C NMR (101 MHz, Methanol-$d_4$, rotamers) $\delta$ 177.0, 176.03 & 176.01, 175.44 & 175.37, 173.9 & 173.7, 173.4 & 173.2, 138.7 & 138.6, 130.24 & 130.22, 129.50 & 129.45, 127.8, 55.9 & 55.7, 54.9 & 54.6, 51.4 & 51.3, 38.5 & 38.4, 30.81 & 30.76, 27.4 & 27.3, 22.51 & 22.48, 17.5 & 17.4; LRMS (ESI⁺): Calcd mass [M+H]⁺: 407.19; mass found: 407.17 [M+H]⁺
IV. Polymerisation procedures

Scheme S9 - Attempted synthesis of polymer 4 by in situ activation with HATU

N-Cbz-phenylalanine (18 mg, 0.060 mmol) and HATU (23 mg, 0.060 mmol) were added to a 25-mL dried Schlenk tube. Under nitrogen atmosphere DMF (0.25 mL) was added, followed by triethylamine (9 µL, 0.06 mmol). The reaction was stirred at room temperature for 1 hour. Analysis of the mixture by ¹H NMR spectroscopy showed 70% conversion of the acid to the activated ester. After that, Co(acac)₂ (15.4 mg, 0.06 mmol) was added to the reaction mixture, followed by DMF (0.75 mL) and methyl acrylate (0.27 mL, 3 mmol). The solution was then deoxygenated three times by freeze-pump-thaw cycle. The solution was cooled to 0 °C, and a 1 M solution of Et₂Zn in hexane (60 µL, 0.06 mmol) was added dropwise. After stirring at 0 °C for 3 h, an aliquot was taken to determine the monomer conversion by ¹H-NMR spectroscopy (14% conversion). A degassed solution of TEMPO in DMF (18.7 mg, 0.12 mmol in 1 mL) was then added into the reaction mixture and stirred at room temperature for 2 h. The resulting PMA was isolated by precipitation with a large amount of water and the polymer was dried under reduced pressure.

Scheme S10 - General procedure for polymerization using isolated TCNHPI esters

N-Cbz-phe-TCNHPI (35 mg, 0.06 mmol) and Co(acac)₂ (46.3 mg, 0.18 mmol) were added to a 25-mL Schlenk tube equipped with a stir bar. Under nitrogen atmosphere, dried DMF (1 mL) was added, followed by methyl acrylate (0.27 mL, 3 mmol). The solution was then deoxygenated three times by freeze-pump-thaw cycle. The solution was cooled to 0 °C, and transferred to a dry microwave tube containing Zn powder (3.9 mg, 0.06 mmol) under nitrogen atmosphere. After stirring at 0 °C for 2 h and at 3-4 °C for 2 h, an aliquot was taken to determine the monomer conversion by ¹H-NMR spectroscopy (88% conversion). A degassed solution of TEMPO in DMF (56.3 mg, 0.36 mmol in 0.5 mL) was then added to the reaction mixture and
stirred at room temperature for 2 h. The resulting PMA was isolated by precipitation with a large amount of water. The resulting polymer then was purified by dissolving in a small amount of THF and precipitated from a large amount of ether (2 times) and hexane (1 time), then dried under vacuum to give polymer 4.

**Removal of metal content**

Polymer 4 (~200 mg) was dissolved in THF (5 mL) and stirred over basic alumina (2 g) overnight. The mixture was filtered, and the alumina washed with additional THF (3 × 5 mL). The combined THF fractions were concentrated under reduced pressure to give purified polymer 4. This material was analysed by ICP-OES, and the cobalt and zinc contents were below the limit of detection for this method (0.005 ppm for Co and 0.001 ppm for Zn corresponding to sample concentrations of < 2 ppm Co and < 0.5 ppm Zn).

**Figure S1 - Kinetic study for polymerization using isolated TCNHPi ester 3**

| No. | Polymerisation time (mins) | Monomer Conv. | $M_{n,GPC}$ | $M_n/M_w$ |
|-----|---------------------------|---------------|-------------|-----------|
| 1   | 60                        | 13%           | 2,256       | 1.35      |
| 2   | 90                        | 33%           | 4,295       | 1.28      |
| 3   | 120                       | 55%           | 5,396       | 1.26      |
| 4   | 150                       | 64%           | 6,683       | 1.26      |
| 5   | 180                       | 74%           | 7,602       | 1.32      |
| 6   | 210                       | 80%           | 8,263       | 1.29      |

**Scheme S11 - Chain Extension experiment**

1. Co(acac)$_2$, Zn, DMF, 0 °C for 3.5 h
2. TEMPO
3. TEMPO, rt for 2 h
\(N\)-Cbz-phe-TCNHPI (70 mg, 0.12 mmol) and Co(acac)\(_2\) (92.6 mg, 0.36 mmol) were added to a 25-mL Schlenk tube equipped with a stir bar. Under nitrogen atmosphere, dried DMF (2 mL) was added, followed by methyl acrylate (0.54 mL, 6 mmol). The solution was then deoxygenated three times by freeze-pump-thaw cycle. The solution was cooled to 0 °C, and transferred to two dry microwave tubes (I and II) containing Zn powder (3.9 mg, 0.06 mmol) under nitrogen atmosphere. After stirring at 0 °C for 2 h and at 3-4 °C for 2 h, an aliquot was taken to determine the monomer conversion by \(^1\)H-NMR spectroscopy (88% conversion). A degassed solution of TEMPO in DMF (56.3 mg, 0.36 mmol in 0.5 mL) was then added to the reaction mixture I and stirred at room temperature for 2 h, while a degassed solution of methyl acrylate (0.54 mL, 6 mmol) was then added to the reaction mixture II and stirred at 6 °C for overnight. An aliquot was taken from mixture II to determine the monomer conversion after chain extension by \(^1\)H-NMR spectroscopy (high conversion). A degassed solution of TEMPO in DMF (56.3 mg, 0.36 mmol in 0.5 mL) was then added to the reaction mixture II and stirred at room temperature for 2 h. The resulting PMA from I and II were isolated separately by precipitation with a large amount of water. The resulting polymer then was purified by dissolving in a small amount of THF and precipitated from a large amount of ether (2 times) and hexane (1 time), then dried under vacuum to give chain extended polymer.

![Figure S2 – GPC trace of chain extension experiment](image-url)

\(M_n = 32,500 \text{ g/mol, } D = 1.45\)

\(M_n = 8,900 \text{ g/mol, } D = 1.18\)
Scheme S12 - Synthesis of Polymer 19 (ω-functionalisation using diarylzinc reagent)

3-N-Boc-4-(2,4,5-trifluorophenyl)butanoic-TCNHPI ester (37 mb, 0.06 mmol) and Co(acac)$_2$ (46.3 mb, 0.18 mmol) were added to a 25-mL Schlenk tube equipped with a stir bar. Under nitrogen atmosphere, dried DMF (1 mL) was added, followed by methyl acrylate (0.27 mL, 3 mmol). The solution then was deoxygenated three times by freeze-pump-thaw cycle. The solution was cooled to 0°C, and transferred to a dry microwave tube containing Zn powder (3.9 mg, 0.06 mmol) under nitrogen atmosphere. After stirring at 0 °C for 2 h and at 3-4 °C for 2 h, an aliquot was taken to determine the monomer conversion by $^1$H-NMR spectroscopy (66% conversion). A degassed solution of organozinc reagent in ether (20 mL 0.016 M, 0.36 mmol) which was synthesised by reported procedure$^1$ was then added to the reaction mixture and stirred at room temperature for overnight. The resulting polymer was isolated by evaporating ether and unreacted monomer using rotary evaporator, followed by precipitation with a large amount of water. The resulting polymer then was purified by dissolving in a small amount of THF and precipitated from a large amount of ether (2 times), followed by purification using GPC preparative (Shepadex), then dried under vacuum to give polymer 19.

$^1$Chisholm, M. H.; Gallucci, J. C.; Yin, H.; Zhen, H., Arylzinc Alkoxides: $[\text{ArZnOCHP}]_2$ and $\text{ArZn}_3(\text{OCHP})_4$ When $\text{Ar} = \text{C}_6\text{H}_5$, p-$\text{CF}_3\text{C}_6\text{H}_4$, 2,4,6-Me$_3\text{C}_6\text{H}_2$, and C$_6$F$_5$. Inorganic Chemistry 2005, 44 (13), 4777-4785.
V. NMR Spectra of activated esters and peptides

Figure S3 - NHPI ester 2 - \(^1\)H NMR (400 MHz, DMSO-\(d_6\))

Figure S4 - NHPI ester 2 - \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\))
Figure S5 - TCNHPI ester 3 - $^1$H NMR (400 MHz, Acetone-$d_6$)

Figure S6 - TCNHPI ester 3 – $^{13}$C NMR (100 MHz, DMSO-$d_6$)
Figure S7 - TCNHPI ester S6 - $^1$H NMR (400 MHz, DMSO-$d_6$)

Figure S8 - TCNHPI ester S6 – $^{13}$C NMR (100 MHz, DMSO-$d_6$)
Figure S9 - TCNHPI ester S7 - $^1$H NMR (400 MHz, DMSO-$d_6$)

Figure S10 - TCNHPI ester S7 - $^{13}$C NMR (100 MHz, DMSO-$d_6$)
Figure S12 - TCNHPI ester S12 - $^1$H NMR (400 MHz, CDCl$_3$)

Figure S13 - TCNHPI ester S12 - $^{13}$C NMR (100 MHz, CDCl$_3$)
Figure S14 - TCNHPi ester 20 - $^1$H NMR (400 MHz, C$_6$D$_6$)

Figure S15 - TCNHPi ester 20 - $^{13}$C NMR (100 MHz, C$_6$D$_6$)
Figure S16 - Peptided S13 - $^1$H NMR (400 MHz, Methanol-$d_4$)

Figure S17 - Peptided S13 - $^{13}$C NMR (101 MHz, Methanol-$d_4$)
Figure S18 - Peptided S14 - $^1$H NMR (400 MHz, Methanol-$d_4$)

Figure S19 - Peptided S14 - $^{13}$C NMR (101 MHz, Methanol-$d_4$)
Figure S20 - Peptided S15 - $^1$H NMR (400 MHz, Methanol-$d_4$)

Figure S21 - Peptided S15 – $^{13}$C NMR (101 MHz, Methanol-$d_4$)
**Figure S22** - $^1$H-NMR of Activated Ester 18 (600 MHz, DMSO)

**Figure S23** $^{13}$C-NMR of Activated Ester 18 (151 MHz, DMSO)
VI. NMR Spectra and GPC traces of polymers

**Polymer 4**

**Polymer 5**

\[ M_{n,NMR} = 21,500 \]
\[ M_{n,GPC} = 9,300 \]
\[ D = 1.15 \]

**Figure S24** - Polymers 4 and 5 (Table 1 Entry 5) \(^1\)H NMR (400 MHz, Acetone-\(d_6\))
**Figure S25** - Polymer 4 $^1$H NMR (400 MHz, Acetone-$d_6$)

Conv. = 88%

$M_n$,$\text{NMR}$ = 8,100

$M_n$,$\text{GPC}$ = 8,900

$D$ = 1.18

$M_n = 8,900$

$M_w/M_n = 1.18$

**Figure S26** - GPC trace of polymer 4
Figure S27 - Polymer 6 $^1$H NMR (400 MHz, Acetone-$d_6$)

$M_n = 15,300$

$M_w/M_n = 1.25$

Figure S28 - GPC trace of polymer 6
**Figure S29** - Polymer 7 $^1$H NMR (400 MHz, Acetone-$d_6$)

$M_n = 8,400$

$M_w/M_n = 1.29$

**Figure S30** - GPC trace of polymer 7
Figure S31 - Polymer 8 $^1$H-NMR (400 MHz, Acetone-d$_6$)

$M_n = 8,900 \text{ g/mol}$

$M_w/M_n = 1.19$

Figure S32 - GPC trace of polymer 8
Figure S33 - Polymer 10 $^1$H-NMR (400 MHz, Acetone-d$_6$)

$M_n$ = 9,700 g/mol
$M_w/M_n$ = 1.24

Figure S34 - GPC trace of polymer 10
Figure S35 Polymer 11 $^1$H-NMR (400 MHz, Acetone-$d_6$)

$M_{n,NMR} = 17,700$

Figure S36 GPC trace of polymer 11

$M_n = 16,700 \text{ g/mol}$

$M_w/M_n = 1.55$
Figure S37 - Polymer 12 $^1$H-NMR (400 MHz, Acetone-$d_6$)

$M_n = 12,200$

$M_w/M_n = 1.19$

Figure S38 - GPC trace of polymer 12
**Figure S39 - Polymer 13 ¹H-NMR (400 MHz, Acetone-\textit{d₆})**

\[
\begin{align*}
M_n &= 12,200 \\
M_w/M_n &= 1.24
\end{align*}
\]

**Figure S40 - GPC trace of polymer 13**
Figure S41 - Polymer 14 $^1$H-NMR (400 MHz, Acetone-$d_6$)

$M_n = 9,600$

$M_w/M_n = 1.28$

Figure S42 - GPC trace of polymer 14
**Figure S43** - Polymer 15 $^1$H-NMR (400 MHz, Acetone-$d_6$)

$M_n = 5,500$

$M_w/M_n = 1.56$

**Figure S44** - GPC trace of polymer 15
Figure S45 - Polymer 16 $^1$H-NMR (400 MHz, DMSO-$d_6$)

$M_n = 7,500$

$M_w/M_n = 1.24$

Figure S46 - GPC trace of polymer 16
Figure S47 - Polymer 17 $^1$H NMR (400 MHz, Acetone-$d_6$)

$M_n = 8,200$
$M_w/M_n = 1.28$

Figure S48 - GPC trace of polymer 17
**Figure S49** - Polymer 19 $^1$H-NMR (600 MHz, Acetone-$d_6$)

**Figure S50** - $^{19}$F-NMR of Polymer 19 in Acetone-$d_6$
Figure S51 - GPC trace of polymer 19

\[ M_n = 12.200 \text{ g/mol} \]
\[ M_w/M_n = 1.42 \]
**Figure S52** - Polymer 21 \(^1\)H NMR (400 MHz, DMSO-\(d_6\))

\[ M_n = 11,600 \]
\[ M_w/M_n = 1.38 \]

**Figure S53** - GPC trace of polymer 21
VII. DOSY NMR experiments

**Figure S54** – DOSY NMR of polymer 4
The diffusion constants from DOSY NMR of Polymer 4, analysed by topspin are: $5.17 \times 10^{-10}$ m$^2$/s for α-endgroup (peak a, 7.25 ppm), $5.08 \times 10^{-10}$ m$^2$/s for bulk polymer (peak c, 3.65 ppm), $5.12 \times 10^{-10}$ m$^2$/s for ω-endgroup (peak f, 1.09 ppm), and $4.44 \times 10^{-9}$ m$^2$/s for residual solvent peak (acetone-$d_6$).

**Figure S55** - Control experiment with activated ester 3 and polymer with non-aromatic endgroup. The diffusion constants from DOSY NMR are: $9.09 \times 10^{-10}$ m$^2$/s for free activated ester (peak a, 7.30 ppm) and $1.77 \times 10^{-10}$ m$^2$/s for bulk polymer (3.65 ppm).
**Figure S56** - The diffusion constants from DOSY NMR of Polymer 17, analysed by tospin are:

- $3.95 \times 10^{-10}$ m$^2$/s for $\alpha$-endgroup (peak $a_1$, 7.26 ppm)
- $3.65 \times 10^{-10}$ m$^2$/s for bulk polymer (peak $b$, 3.65 ppm)
- $4.28 \times 10^{-10}$ m$^2$/s for $\omega$-endgroup (peak $a_2$, 6.91 ppm)
- $3.61 \times 10^{-9}$ m$^2$/s for residual solvent peak (acetone-$d_6$).
**Figure S57** - The diffusion constants from DOSY NMR of polymer 19 are 3.42 × 10⁻¹⁰ m²s⁻¹ for the α-end group (signal a, 7.2 ppm), 3.76 × 10⁻¹⁰ m²s⁻¹ for ω-end group (signal e, 7.7 ppm) and 2.99 × 10⁻¹⁰ m²s⁻¹ for the polymer backbone (signals b and c). Solvent (Acetone) = 4.15 × 10⁻⁹ m²s⁻¹.
VIII. MALDI-TOF MS Data

Polymers 4 and 5 (using ZnEt₂ as a reductant)

Figure S58 – MALDI-MS of polymers 4 and 5 (using ZnEt₂ as a reductant)
VIII. ESI-TOF MS Data

1293.61 calcd. for n=10, found 1293.60 [M+Na]^+

Figure S59 – ESI-MS of polymer 4 (using Zn as a reductant, quenched with TEMPO at 13% conversion)
Figure S60 – ESI-MS of polymer 4 with no TEMPO quenching. (a) 1:3:3 ratio of activated ester:cobalt:zinc, stirred at 3-6 °C overnight in DMF, (b) 1:3:3 ratio 1:3:3 ratio of activated ester:cobalt:zinc, stirred at 3-6 °C overnight in DMF-d7,
**Figure S61** – Polymer 17 quenched with organozinc reagent at low conversion

The ω-end containing fragment was observed, consistent with the known fragmentation pattern for PMA. See: K. Chaicharoen, M.J Police, A. Singh, C. Pugh, C. Wesdemiotis, *Anal Bioanal Chem.*, 2008, **392**, 595–607; E. Altuntas, A. Kreig, A. Baumgaertel, A. Crecelius, U. Schubert, *Polym. Chem.* 2013, **51**, 1595-1605.

1492.61 calcd. for n=12, found 1492.67 [M+Na]$^+$
IX – Polymer graphene conjugation procedure

*N*-Cbz-phe-TCNHPI (35 mg, 0.06 mmol) and Co(acac)₂ (46.3 mg, 0.18 mmol) were added to a 25-mL Schlenk tube equipped with a stir bar. Under nitrogen atmosphere, dried DMF (1 mL) was added, followed by methyl acrylate (0.27 mL, 3 mmol). The solution was then deoxygenated three times by freeze-pump-thaw cycle. The solution was cooled to 0 ºC, and transferred to a dry microwave tube containing Zn powder (3.9 mg, 0.06 mmol) under nitrogen atmosphere. After stirring at 0 ºC for 2 h and at 3-4 ºC for 2 h, the solution was transferred by cannula to a flask containing “single layer graphene” (20 mg, ACS materials, 0.6-1.2 nm thickness, 400-1000 m²/g surface area) and stirred overnight at room temperature.

A solution of aqueous HCl (1.0 M, 3 mL) was added, and the mixture stirred at room temperature for 3 hours. The graphene material was isolated by centrifugation (7000 rpm, 2 minutes) and resuspended in DMF (15 mL). The graphene material was again isolated by centrifugation, and this procedure repeated with H₂O (15 mL × 2), MeOH(15 mL ×2) and EtOAc (15 mL ×3) to give the conjugate material as a black solid.

X – Protein conjugation method and LC/MS data

Methods:

Protein Conjugation:

FN3Con anti-Lys.V2 was expressed and purified as per the previous report (B. T. Porebski, A. M. Buckle et Al. Protein Eng. Des. Sel. 2016, 541-550), then repurified into Reaction Buffer (0.1 M Sodium Bicarbonate, pH8.5). Polymer 22 was dissolved in MilliQ water in an Eppendorf tube, then FN3Con anti-Lys.V2 sample was added to a final polymer:protein ratio of 50:1. The reaction mixture was mixed by inversion and incubated for 16 hours at 60 ºC in a heatblock, the reaction mixture was then quenched with Quench buffer (1M Glycine-HCL, pH 7.4). Samples were diluted 5 times with reaction buffer and separated with SDS-PAGE. After gel staining and imaging, protein bands were excised and analysed by LC/MS.

Mass spectrometric acquisition

Nano LC System: Dionex Ultimate 3000 RSLCnano
Mass spectrometer: QExactive Plus 2 (Thermo Scientific)
Analytical column: Acclaim PepMap RSLC (75 μm x 50 cm, nanoViper, C18, 2 μm, 100Å; Thermo Scientific)
Trap column: Acclaim PepMap 100 (100 μm x 2 cm, nanoViper, C18, 5 μm, 100Å; Thermo Scientific)
Acquisition method: 68_30_DDA_LowComplex

Number of searches: 1
Search engine: Byonic (ProteinMetrics)
Data base: Uniprot human incorporating supplied sequence (FN3Con-anti-lysozyme)
Protein FDR cutoff: 1%
Fixed modification: Carbamidomethylation
Variable modification: Oxidation at M

**LC/MS results:**

*Measured peptides:*

| Reference peptide (GYP) | Reference peptide (VTD) | First lysine (EAG) |
|-------------------------|-------------------------|--------------------|
| MASPSPPGNL RVTDTVSTSV TLSWRGYPWA TYYGVEYREAA GGEWQVFTM | | |
| PGDLSHRYTV TGLKPGTEYE FRVYAVNRVG RTFDTGPSSS VSVTTGSHHH | Loss peptide (QVF) | Second lysine (YTV) |

For this experiment we identified two peptides to act as normalisation markers between LC/MS experiments with different total masses of protein (Peptides VTD and GYP). The small size and presence of the first lysine peptide (EAG) was validated by the presence of the Loss peptide (QVF), which would also decrease upon conjugation as the modified Lysine of peptide EAG would be protected from cleavage. The Second lysine fragment (YTV) displayed clear changes in presence between samples.

**LC/MS chromatograms:**

10kDa
Area of peaks in liquid chromatography (matched to peptides by MS):

- Raw peak area of fragments used for normalisation

From these results we chose to use the VTD reference peptide, as it followed a clear trend that more closely qualitatively matched the change in other peptide presence.

- Raw peak area of lysine containing peptides

All peptides show a similar change in peak area between sample, although this will mostly account for changes in total mass between samples.
- Normalised peak area of lysine-containing peptides (Peak Area/Area of VTD peptide)

Normalisation allows us to calculate a change in the lysine peptides relative to the change occurring in reference peptides.

- Peak area of peptide fragments after normalisation to a reference, expressed as % of area in the 10kDa band.