Electronic Supplementary Material (ESI) for Polymer Chemistry.

SUPPORTING INFORMATION

Grafting-From Protein's: Utilization of a Common Amino Acid Residue as a New Grafting Site

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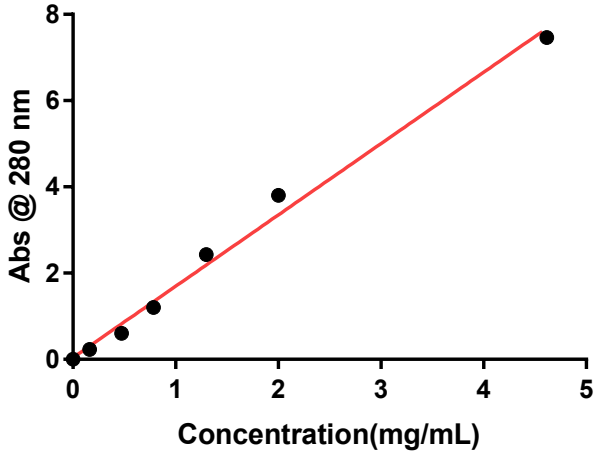
Methods

Determination of the concentration of Lipase TL-polymer hybrids. To determine the concentration of enzyme after grafting, the absorbance at 280 nm and Direct Detect® FTIR spectrometer were used to build a calibration curve below, with a 280 nm absorbance value of 0.74 mg/mL = 1 AU (absorbance unit).



Determination of the experimental M_n (M_{nexpt}) by UV-Vis Spectroscopy and Direct Detect® FTIR spectrometer

The experimental M_n was determined by measuring the total concentration of the bio hybrid in solution using a Direct Detect IR spectrometer. The Direct Detect instrument used to measure the concentration of the amide bond from the IR peak (both the polymers and proteins have amide bonds in their backbones). The polymer concentration was obtained by subtracting the mass of protein in solution.

(1) $W_{polymer}{}^{a} = [PPH]^{b} - [protein]^{c} = [polymer]$ (mg/mL)

[a]Mass fraction polymer. [b]Concentration of protein polymer hybrid was determined using DirectDetect IR spectrophotometer. [c]Concentration determined using UV-vis, absorption at 280 nm.

The mass of the protein in solution was determined using UV-vis spectroscopy, the polymer does not absorb at 280 nm in the UV-vis spectrum. Once the mass of the polymer in solution was obtained the number of moles of both polymer and protein were determined and moles polymer/moles protein/number of *i*BBr (N_{iBBr}) initiators per protein gave the average degree of polymerization (DP) for each grafted polymer.

(2) $n_{polymer} = [polymer]/MW_{monomer}$ (mmol)

$n_{protein} = [polymer]/MW_{TL}$ (mmol)

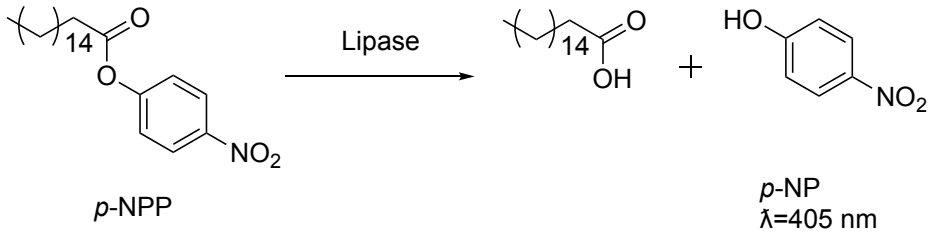
(3) $DP(per\ iBBr) = n_{polymer} / n_{protein}/N_{iBBr}$

Multiplying the DP by the molecular weight of the monomer gives the number average molecular weight of single polymer.

(4) $M_{n\ (polymer)} = DP \times Mw_{monomer}$

(5) $M_{n,expt} = M_{n(polymer)} \times N_{iBBr} + MW_{TL\text{-}iBBr}$

Assay of lipolytic activity and thermal stability of lipase TL and lipase-polymer hybrids. Lipolytic activity was determined spectrophotometrically by using *p*-nitrophenyl palmitate (*p*-NPP) as substrate.

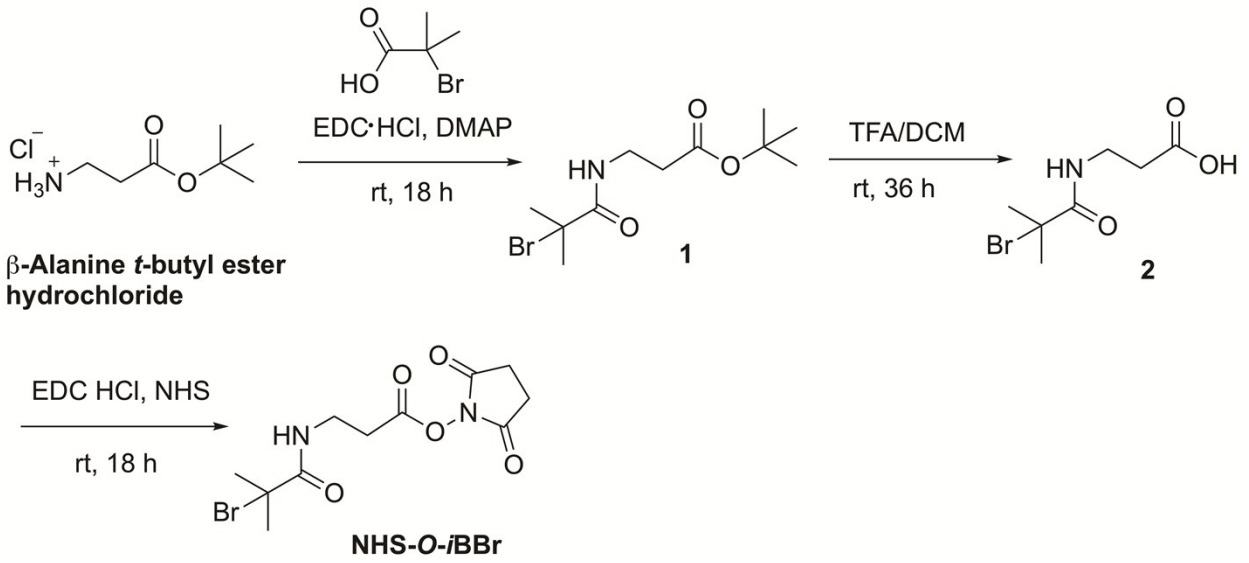


p-NPP

p-NP
λ=405 nm

Synthesis and Characterization

Synthesis of NHS-O-iBBr ATRP initiator

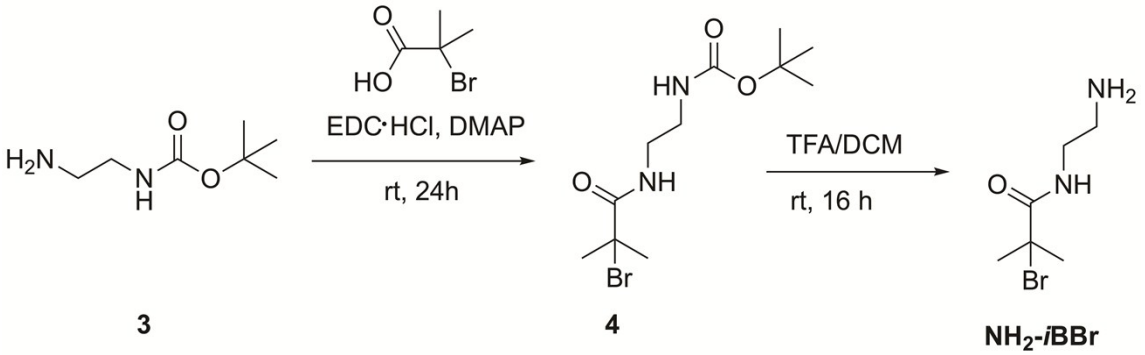


Synthesis of 1. β-Alanine *t*-butyl ester hydrochloride (1.13 g, 6.25 mmol), *i*BBr-COOH (1.87 mg, 11.25 mmol, 1.8 eq) and DMAP (38 mg, 0.31 mmol, 0.05 eq) were dissolved in dichloromethane (30 mL). EDC (1.32 g, 6.87 mmol, 1.1 eq) was added to the reaction mixture at 0 °C. After the addition, the reaction was stirred at room temperature overnight. The reaction mixture was then washed with 1N NaOH, water and brine. The organics were dried over $MgSO_4$, filtered and the solvent was evaporated under vacuum; yield (1.2 g, 66%). ^{1}H-NMR (500 MHz; $CDCl_3$): δ 7.20 (bs, 1H), 3.44 (q, J = 6.0 Hz, 2H), 2.43 (t, J = 6.0 Hz, 2H), 1.88 (s, 6H), 1.42 (s, 9H).

Synthesis of 2. Compound **1** (1.0 g, 3.4 mmol) was dissolved in 50 ml of dichloromethane and trifloroacetic acid (TFA, 2.7 ml, 3.4 mmol) was added dropwise to the reaction mixture. The reaction was stirred for 36 hours, and subsequently extracted 3 times with 30 ml of water and once with brine. The organic phase was dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure; yield (0.8 g, 98%). ^{1}H-NMR (500 MHz; DMSO-d_6): δ 13.10 (bs, 1H), 8.10-8.08 (m, 1H), 3.31 (q, J = 6.4 Hz, 2H), 2.42 (t, J = 7.2 Hz, 2H), 1.87 (d, J = 0.5 Hz, 6H).

Synthesis of NHS-*O-i*BBr. Compound **2** (0.4 g, 1.3 mmol), EDC-HCl (0.25 g, 1.3 mmol) and NHS (0.15 g, 1.3 mmol) were dissolved in 10 ml of DCM and stirred for 18 hours. 40 ml of DCM and 30 ml of water were then added to the reaction mixture and stirred for 10 minutes. The organic phase was separated and the aqueous phase was washed 3 times with 20 ml of DCM. The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure; yield (0.39 g, 90%). ^{1}H-NMR (500 MHz; $CDCl_3$): δ 7.19 (bs, 1H), 3.66 (q, J = 6.0 Hz, 2H), 2.88-2.85 (m, 6H), 1.93 (s, 6H).

Synthesis of NH_2-iBBr ATRP initiator



Synthesis of 4. *tert*-Butyl (2-aminoethyl)carbamate, **3** (5.0 g, 31.2 mmol), iBBr-COOH (7.8 g, 46.8 mmol, 1.5 eq) and DMAP (190.58 mg, 1.56 mmol, 0.05 eq) were dissolved in dichloromethane (150 mL). EDC (7.19 g, 37.5 mmol, 1.1 qe) was added at 0 ^{o}C. The reaction was stirred at room temperature for 24 h. Then the reaction mixturet wast washed with 1N NaOH, water and brine. The organics were dried over $MgSO_4$, filtered and the solvent was evaporated under vacuum; yield (5.9 g, 62%). ^{1}H-NMR (500 MHz; $CDCl_3$): δ 7.25 (bs, 1H), 4.96 (bs, 1H), 3.36-3.28 (m, 4H), 1.92 (s, 6H), 1.42 (s, 9H).

Synthesis of NH_2-*i*BBr ATRP initiator. Compound **4** (2 g, 6.49 mmol) was dissolved in 100 mL of a 8:2 mixture of DCM and TFA. The resulting solution was stirred at room temperature for 16 h. After removal of the solvent, the reaction mixture was washed with 1N NaOH, water and brine. The organics were dried over $MgSO_4$, filtered and the solvent was evaporated under vacuum; yield (1.50 g, 72%). ^{1}H-NMR (500 MHz; DMSO-d_6): δ 8.24 (t, J = 5.5 Hz, 1H), 7.89 (bs, 2H), 3.35 (q, J = 6.2 Hz, 2H), 2.89 (q, J = 6.1 Hz, 2H), 1.88 (s, 6H).

Supplemental Figures and Tables

Table S1. Condition for the synthesis of TL-K-*i*BBr, modification of lysine residue of TL.

Entry	TL (mg/mL)	NHS-*O*-*i*BBr (equiv.)	Number of *i*BBr moieties
1	10	100	3
2	10	200	3
3	5	200	3

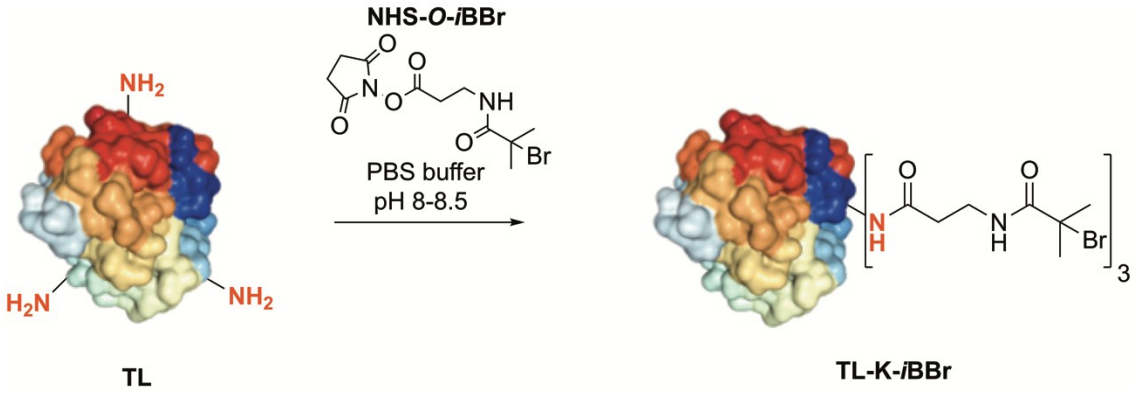
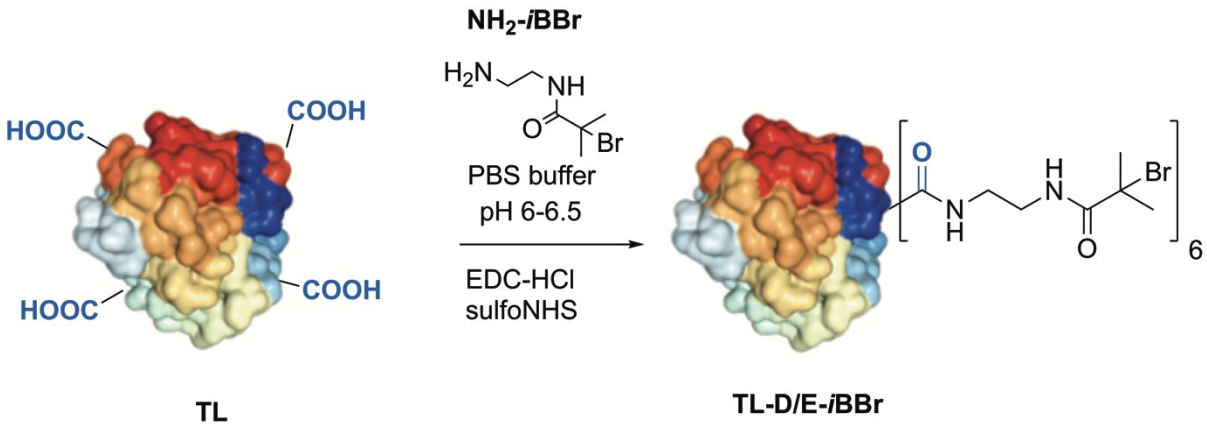


Table S2. Condition for the synthesis of TL-D/E-*i*BBr, modification of aspartic and glutamic residue of TL.

Entry	TL (mg/mL)	NH_2-*i*BBr (equiv.)	sulfo-NHS	EDC-HCl (mM)	Number of *i*BBr moieties
1	10	100	5	0.05	2
2	5	200	5	0.05	6



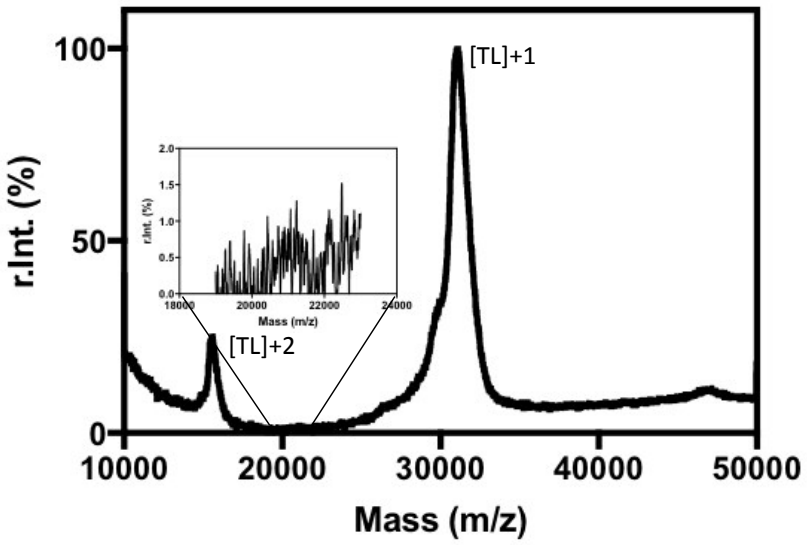


Figure S1. MALDI-ToF spectrum of TL lipase.

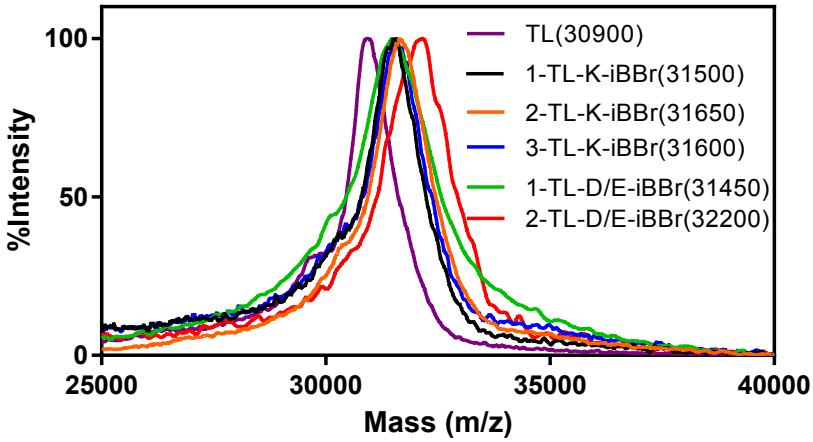


Figure S2. MALDI-ToF MS data for TL and TL-*i*BBr after different reaction conditions.

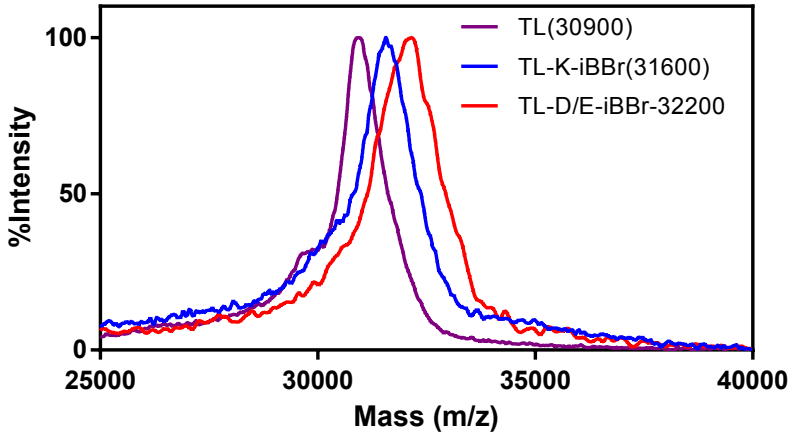


Figure S3. MALDI-ToF MS data for TL and TL-*i*BBr.

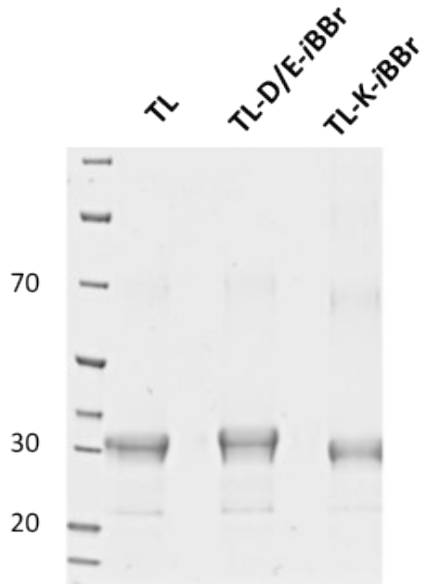


Figure S4. SDS-PAGE analysis of TL lipase and *i*BBr initiators.

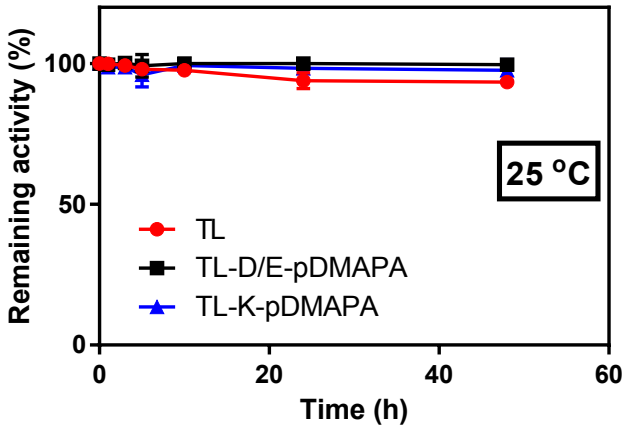


Figure S5. Time course of thermal inactivation of lipase TL at 25 °C, in comparison to and lipase polymer hybrids (TL-D/E-pDMAPA, TL-K-pDMAPA). Thermal stability was investigated by monitoring the concentration of p-NP formed after the incubation at a given time compared to the activity at zero time of incubation at given temperature (expressed as a percentage). Each point is the average of triplicates ±SD.

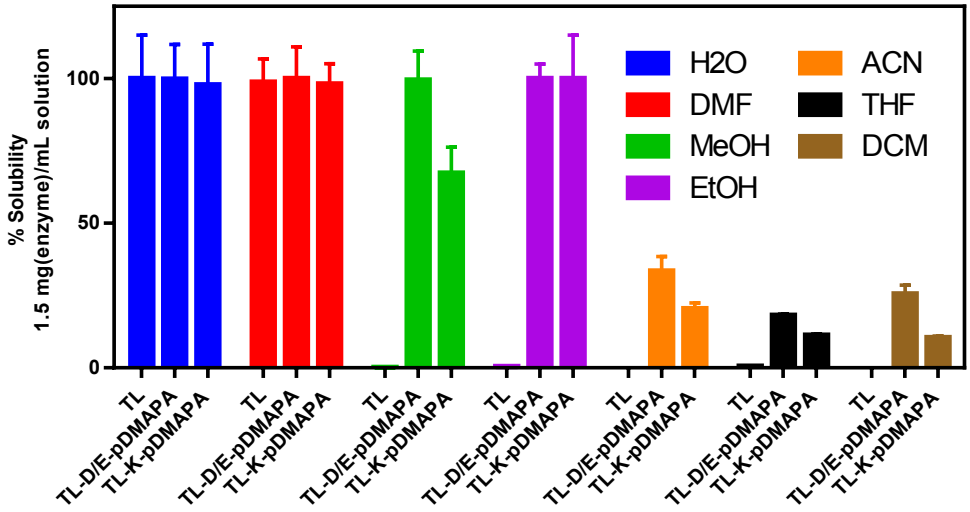


Figure S6. Comparative solubility of lipase TL (1.5 mg/mL), TL-K-pDMAPA (3.2 hybrid mg/mL, 1.5 enzyme mg/mL) and TL-D/E-pDMAPA (3.6 hybrid mg/mL, 1.5 enzyme mg/mL), was determined by measuring the concentration of the sample solution using Direct Detect® FTIR spectrometer and comparing it to solubility in aqueous media.

Table S3. Lipolytic activity of TL lipase and TL biohybrids.

	Lipase activity (μmol *p*-NP min^{-1} mg^{-1})
TL	132.47 ± 9.15
TL-D/E-*i*BBr	130.12 ± 1.90
TL-K-*i*BBr	127.55 ± 5.13
TL-D/E-pDMAPA	187.55 ± 6.34 - 197.75 ± 11.60
TL-K-pDMAPA	141.72 ± 3.13 – 148.34 ± 4.17