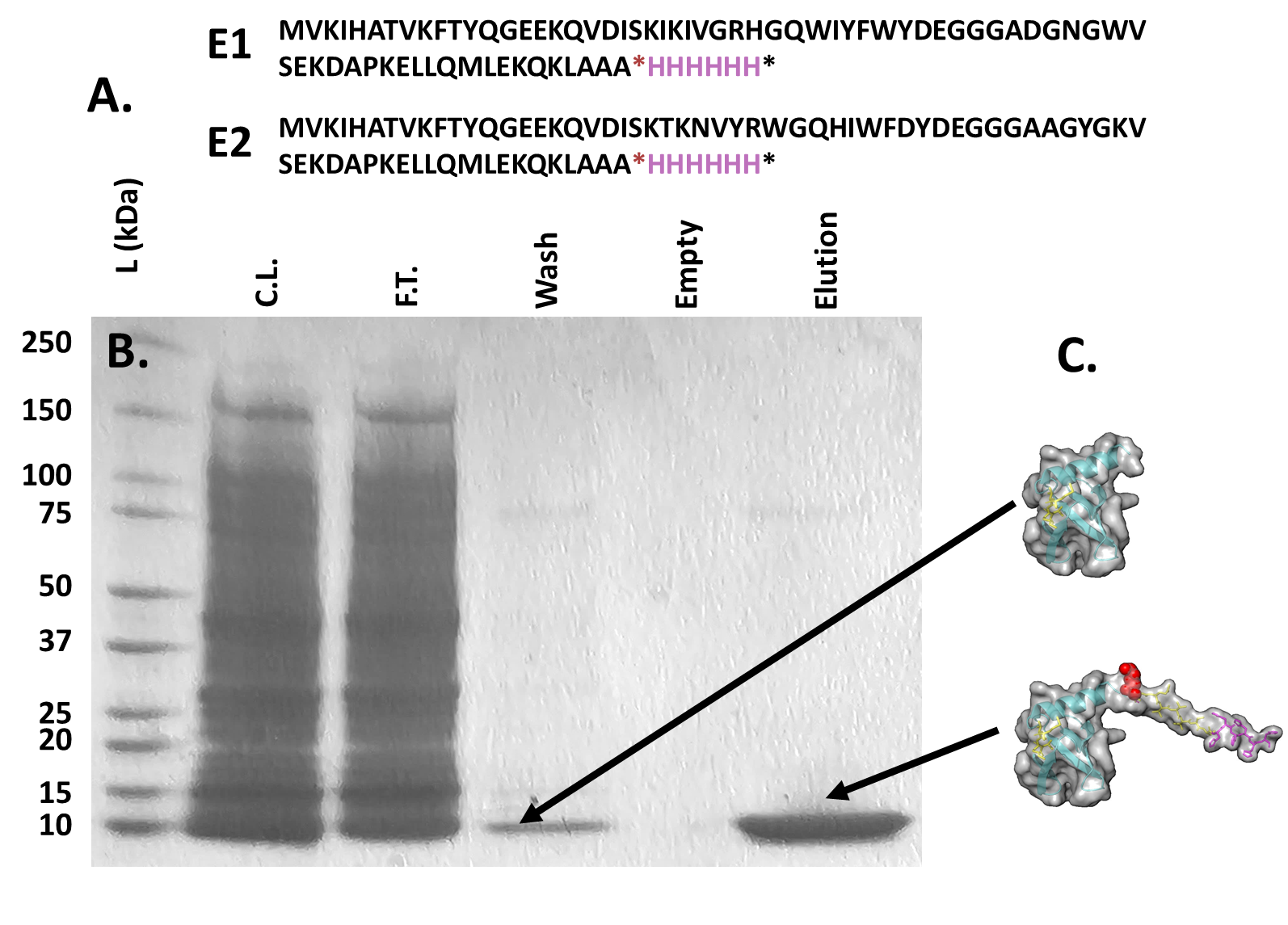
**1. MATERIALS AND INSTRUMENTATION**

**Materials.** **N3-PEG-NH2** was synthesized according to literature procedures1,2 using PEG 400 purchased from Alfa Aesar. Poly(ethylene glycol) methyl ether methacrylate (Mn = 500), 2,2-azobis(2-methylpropionitrile) (AIBN), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid, benzyl methacrylate, poly(ethylene glycol) methyl ether azide (Mn = 1000), Zonyl FS-300, Tween 20, sodium ascorbate, 1,4-dioxane, diethylbenzene (DEB), N,N-dimethylformamide (DMF), hexanes, tetrahydrofuran (THF), anti-mouse IgG (whole molecule)-FITC antibody, and human serum (from human male AB plasma, USA origin, sterile-filtered) were purchased from Sigma-Aldrich. Pentafluorophenyl methacrylate and 2-(trifluoromethyl)-3-ethoxydodecafluorohexane (HFE-7500) were purchased from Synquest. DBCO-amine was purchased from BroadPharm. 2-hydroxyethyl acrylate and cetyltrimethylammonium bromide (CTAB) were purchased from Alfa Aesar. 4,4'-difluorobenzophenone and dimethyl sulfoxide (DMSO) were purchased Oakwood Chemical. Mouse anti-human IL-6 capture antibody (Human IL-6 DuoSet ELISA) was purchased from R&D Systems. Recombinant human IL-6 was purchased from RayBiotech. Human CRP was purchased from Innovative Research. IL-6 monoclonal antibody (MQ2-13A5), eBioscience™ and goat anti-rat IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor™ 594 were purchased from ThermoFisher. Azidobutyric acid NHS ester and DBCO NHS ester were purchased from Lumiprobe. Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) was purchased from Ambeed. Copper sulfate was purchased from Mallinckrodt Baker, Inc. 5’-hexynyl-AAA AA-FAM-3’ was purchased from Integrated DNA Technologies. PBS tablets, ethanol, and nuclease free water were purchased from VWR. Mili-Q water from Barnstead Nanopure Water System (Thermo Fisher Scientific) was used for the preparation of the buffer for the emulsion continuous phases. All reagents and solvents were used as received without further purification unless otherwise stated. Inhibitor was removed from poly(ethylene glycol) methyl ether methacrylate, pentafluorophenyl methacrylate, benzyl methacrylate, and 2-hydroxyethyl acrylate using basic alumina from Sigma-Aldrich prior to use.

**Cell Culture and Protein Purification.** Transformed cells were cultured in Luria Broth (LB) media with appropriate antibiotics. Cell were grown until mid-log phase (OD600=0.5-0.8) while shaking (220 rpm, 37 °C). Cells were induced by the addition of 60 µM IPTG and 1 mM 4AZP generated as described3 and shaken overnight (220 rpm, 17 °C). Cells were then pelleted (4200 rpm, 20 min) and resuspended in lysis buffer (100 mM HEPES pH 8.0, 300 mM NaCl, 10 mM imidazole, 30mL/L of growth) and frozen until protein purification.

Frozen cell suspensions were thawed at room temperature. Lysis was performed via incubation with lysozyme (0.02 g/L growth) while vigorously stirring at room temperature for 30 minutes, followed by sonication (60 sec on, 60 sec off, 3 times while on ice). Lysates were clarified via centrifugation (20,000 x g, 60 min, 4°C) followed by syringe filtration (0.22 micron). Clarified lysates were purified via gravity using HisPURE Ni2+NTA resin. After loading, resin was washed with 20x column volumes of Lysis buffer, and eluted using 3x column volumes of elution buffer (100 mM HEPES pH 8.0, 150 mM NaCl, 250 mM imidazole). Proteins were buffer exchanged into storage buffer (20 mM HEPES pH 8.0, 100 mM NaCl) via spin concentrator (Amicon Ultra 3kDa MW cutoff) and flash frozen. Protein concentration was determined by Bradford assay, and protein purity was determined via SDS-PAGE. Example SDS-PAGE gel is shown in Figure S2. Proteins were further purified for assays comparing E1/E2 to commercial antibodies via Size Exclusion Chromatography (SEC) at 4°C (HiLoad® 16/600 Superdex® 200 pg). The SEC column was equilibrated with 400 mL storage buffer at 1.5ml/min, and proteins were loaded via syringe injection. Fractions containing E1/E2 were concentrated as previously described.



**Figure S2.** **A.** Representative sequence of E1. non-native stop codon represented as red \*, native stop as black \*, 6X His tag shown in purple. **B.** SDS-PAGE gels of IMAC purification of rcSso7d. Lanes contain clarified lysate (C.L), flow-through of nickel column (F.T.), wash, an empty lane, and elution of the purified protein. **C.** Structural representation of rcSsso7d synthesis projects with truncated product (top) lacking 6xHis tag (purple sticks) and full length protein with 4AZP incorporated (bottom, red balls).

***E. coli* expression plasmid design and ncAA incorporation.** Plasmids for bacterial expression were generated by Genscript. Amino acid sequences for two clones, which form a sandwich pair originally titled “clone2” (E1) and “clone8,” (E2) were provided to Genscript for codon optimization, synthesis, and cloning into the bacterial expression plasmid pRSF-Duet. The original sequences were modified to include an internal amber (TAG) stop codon at the original stop codon, the short spacer LAAA, followed by a hexa-Histidine tag terminated by the ochre (TAA) stop codon. The full sequences for each clone can be found in Figure S2.

To functionalize the described rcSso7d proteins, BL21(DE3) cells were co-transformed with one of the described rcSso7d plasmids alongside the pDULE plasmid previously described,4 which encodes an engineered tRNA that pairs with the amber stop codon and a tRNA synthetase that loads the engineered tRNA with 4-AzidoPhenylalanine (4AZP). This methodology allows the site-specific functionalization at any internal TAG stop codon of proteins with a free azide. This free azide can be used for orthogonal attachment to covalently bond 4AZP containing proteins to various substrates using alkyne azide click chemistry. Transformed cells were cultured, induced, and lysed and the proteins were purified according to procedures stated in the experimental section. A portion of all proteins purifications was labeled with FITC NHS esters to verify attachment to droplets.

**rcSso7d NHS-Ester labeling.** Proteins were incubated with FITC NHS ester dye at a ratio of 7:1 (dye:theoretical lysines/protein) at room temperature while rocking for 30 minutes. Unbound dye was separated from dyed proteins via gel filtration spin columns (BioGel650).

**Synthesis of Azido-Modified Anti-IL-6 Antibody.** IL-6 monoclonal antibody (MQ2-13A5) was buffer exchanged using a spin desalting column (Zeba™ Spin Desalting Columns, 7K MWCO, ThermoFisher) with 20 mM HEPES pH 8.0, 100 mM NaCl. The antibody was then concentrated using a spin concentrator (Amicon Ultra 30kDa MW cutoff, Millipore Sigma) to 10.85 μM (1.62 mg/mL); the protein concentration was determined using a Bradford assay. 26 μL of concentrated anti-IL-6 antibody, 1.4 μL of a 1.6 mM solution of azidobutyric acid NHS ester in DMSO, and an additional 1.5 μL of DMSO was combined in a microcentrifuge tube. The reaction was shaken on a tilter (lowest speed) at room temperature for ~4 h. Unreacted NHS ester was removed using a spin desalting column.

**Synthesis of DBCO-Modified Anti-IL-6 Antibody.** 18 μL of ~3.3 mg/mL of mouse anti-human IL-6 capture antibody dissolved in 20 mM HEPES pH 8.0, 100 mM NaCl and 2 μL of a 1.6 mM solution of DBCO-NHS in DMSO were combined in a microcentrifuge tube. The reaction was shaken on a tilter (lowest speed) at room temperature for ~4 h. Unreacted NHS ester was removed using a spin desalting column.

**General Procedure for Secondary Antibody Experiments.** 7.5 μL of antibody-conjugated droplets were transferred to 200 μL of continuous phase. Then either 2.4 μL of anti-mouse secondary antibody (FITC) or 1 μL of anti-rat secondary antibody (Alexa Fluor™ 594) was added to the droplet solution. The solution was shaken on a tilter (lowest speed) for ~2 h and solvent exchanged three times prior to imaging.

**3. POLYMER SYNTHESIS AND CHARACTERIZATION**

**Scheme S1.** Synthetic scheme of **PDBCO-PPEG-*b*-PBn**.





**PPFP-PPEG-*b*-PBn.** (1) Pentafluorophenyl methacrylate (0.99 g, 3.93 mmol), poly(ethylene glycol) methyl ether methacrylate (1.00 g, 2.01 mmol), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (0.11 g, 0.41 mmol), and AIBN (8.32 mg, 0.05 mmol), and 1,4-dioxane (3 mL) were added to a 10 mL Schlenk flask. The flask was deoxygenated via three freeze-pump-thaw cycles and flushed with argon. The reaction was heated at 75 °C overnight. The reaction mixture was cooled down to room temperature and then quenched with liquid nitrogen. The mixture was precipitated into hexanes, forming a gel. The hexanes was decanted and the polymer was dried under vacuum, resulting in a pink gel, **PPFP-PPEG**, which was used without further purification. (2) **PPFP-PPEG** (0.49 g, 0.08 mmol), benzyl methacrylate (1.17 g, 6.64 mmol), and AIBN (2.6 mg, 0.016 mmol), and 1,4-dioxane (3 mL) were added to a 10 mL Schlenk flask. The flask was deoxygenated via three freeze-pump-thaw cycles and flushed with argon. The reaction was heated at 70 °C for 3 days. The reaction mixture was cooled down to room temperature and then quenched with liquid nitrogen. The mixture was precipitated into hexanes. The resulting polymer was redissolved using THF and precipitated in hexanes two more times. The polymer was dried in a vacuum oven at 60 °C overnight, resulting in a pink solid (1.4 g, 84%). 1H NMR (400 MHz, CD2Cl2) *δ* 7.41-7.19 (m, 5H; benzyl CH), 4.99-4.81 (m, 2H; benzyl CH2), 4.33-3.96 (m, 0.2H), 3.82-3.37 (m, 2.5H), 3.37-3.24 (m, 0.2H; PEG-CH3), 2.68-1.61 (m, 2.5H), 1.60-0.54 (m, 4.2H). 19F NMR (376 MHz, CD2Cl2) *δ* -150.20 (1F), -151.64 (1F), -158.29 (1F), and -162.90 (2F). SEC (PS standards): *M*n = 14.5 kDa, *Ð* = 1.24.



**PDBCO-PPEG-*b*-PBn. PPFP-PPEG-*b*-PBn** (79.2 mg, 0.005 mmol), DBCO-amine (20.5 mg, 0.074 mmol), 2-hydroxyethylacrylate (16 μL, 0.153 mmol), and DMF (0.7 mL) were combined in a round bottom flask. The reaction mixture was heated at 50 °C overnight. Upon cooling to room temperature, the mixture was precipitated into cold ethanol. The polymer was dried under vacuum, resulting in a white solid (34 mg, 42%). 1H NMR (400 MHz, CD2Cl2) *δ* 7.44-7.18 (m, 5H, benzyl CH), 5.02-4.81 (m, 2H, benzyl CH2), 4.28-3.99 (m, 0.1H), 3.84-3.38 (m, 1.6H), 3.38-3.28 (m, 0.1H), 2.53-0.50, 6.5H). 19F NMR (471 MHz, CD2Cl2) *δ* -149.94 (1F), -151.55 (1F), -158.35 (1F), -162.85 (1F). SEC (PS standards): *M*n = 16.3 kDa, *Ð* = 1.18.

**Scheme S2.** Synthetic scheme of **PN3-PPEG-*b*-PBn**.





**PN3-PPEG-*b*-PBn.** **PPFP-PPEG-*b*-PBn** (300 mg, 0.021 mmol), **N3-PEG-NH2** (117 mg, 0.28 mmol), and DMF (2.4 mL) were combined in a round bottom flask. The reaction mixture was heated at 50 °C overnight. Upon cooling to room temperature, the mixture was precipitated into cold ethanol. The resulting polymer was redissolved using THF and precipitated into cold ethanol. The polymer was dried under vacuum, resulting in an off-white solid (115 mg, 36%). 1H NMR (500 MHz, CD2Cl2) *δ* 7.40-7.21 (m, 5H; benzyl CH), 5.02-4.81 (m, 2H; benzyl CH2), 4.27-4.01 (m, 0.1H), 3.72-3.29 (m, 2.5H), 2.53-1.22 (m, 2.8H), 1.16-0.58 (m, 3.3H). SEC (PS standards): *M*n = 19.4 kDa, *Ð* = 1.23.

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