Supporting Information

Efficient Sortase-Mediated Ligation using a Common C-terminal Fusion Tag

Sierra A. Reed, David A. Brzovic, Savanna S. Takasaki, Kristina V. Boyko, John M. Antos*

Department of Chemistry, Western Washington University, 516 High Street, Bellingham, WA, 98225, United States

*Corresponding author: john.antos@wwu.edu

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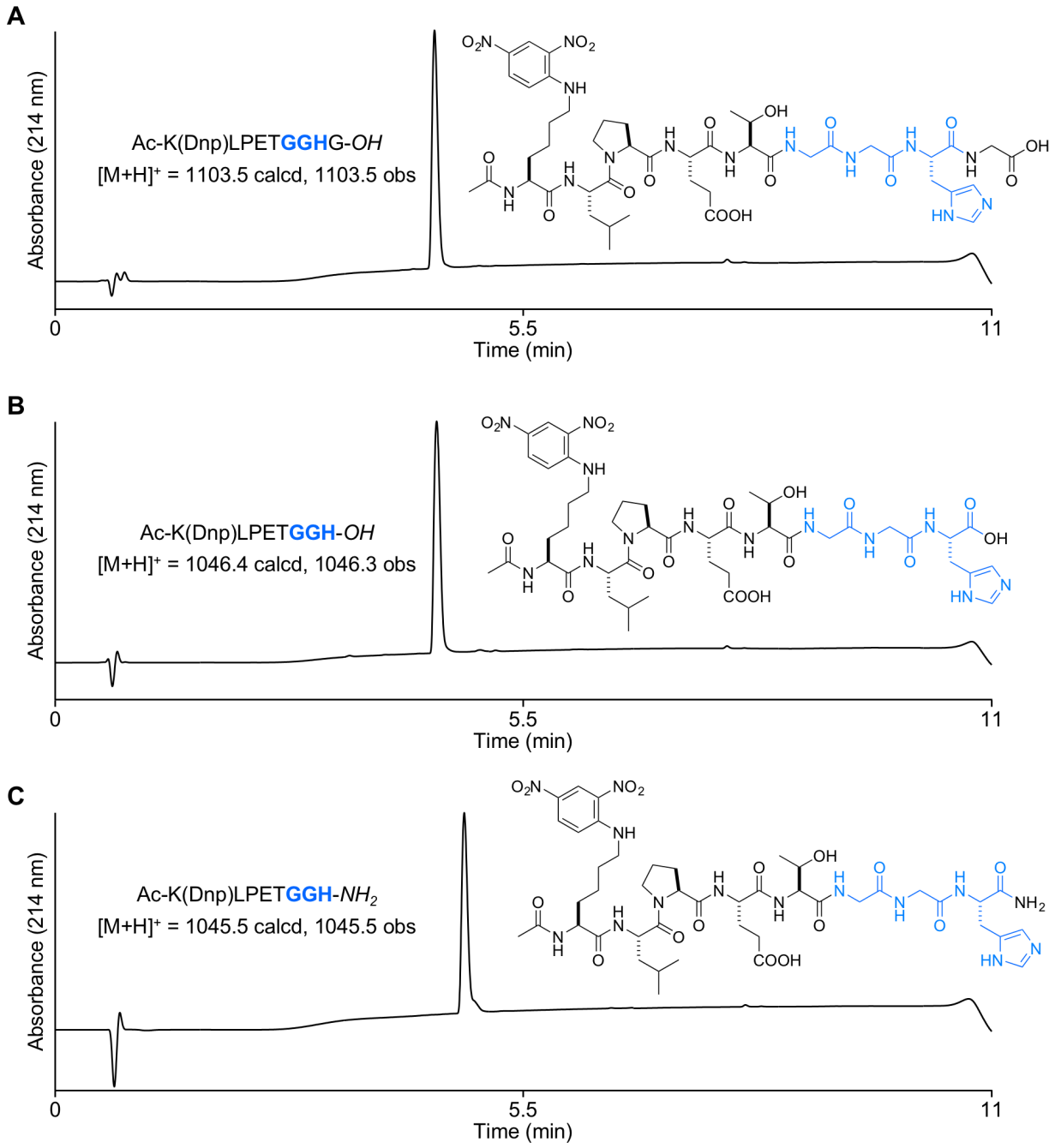


Figure S1. RP-HPLC and LC-ESI-MS characterization of purified (**A**) Ac-K(Dnp)LPETGGHG-*OH*, (**B**) Ac-K(Dnp)LPETGGH-*OH*, and (**C**) Ac-K(Dnp)LPETGGH-*NH*$_2$. All calculated values for [M+H]$^+$ are monoisotopic molecular weights. RP-HPLC conditions for panels **A** and **B**: Phenomenex Kinetex® 2.6 µm C18 100 Å column (100 x 2.1 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic acid) mobile phase at 0.4 mL/min, method: hold 10% MeCN 0.0-1.0 min, linear gradient of 10-90% MeCN 1.0-7.0 min, hold 90% MeCN 7.0-9.0 min, linear gradient of 90-10% MeCN 9.0-9.1 min, re-equilibrate at 10% MeCN 9.1-12.0 min). RP-HPLC conditions for panel **C**: Phenomenex Kinetex® 2.6 µm C18 100 Å column (100 x 2.1 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic acid) mobile phase at 0.4 mL/min, method: hold 5% MeCN 0.0-1.0 min, linear gradient of 5-90% MeCN 1.0-7.0 min, hold 90% MeCN 7.0-9.0 min, linear gradient of 90-5% MeCN 9.0-9.1 min, re-equilibrate at 5% MeCN 9.1-11.5 min.

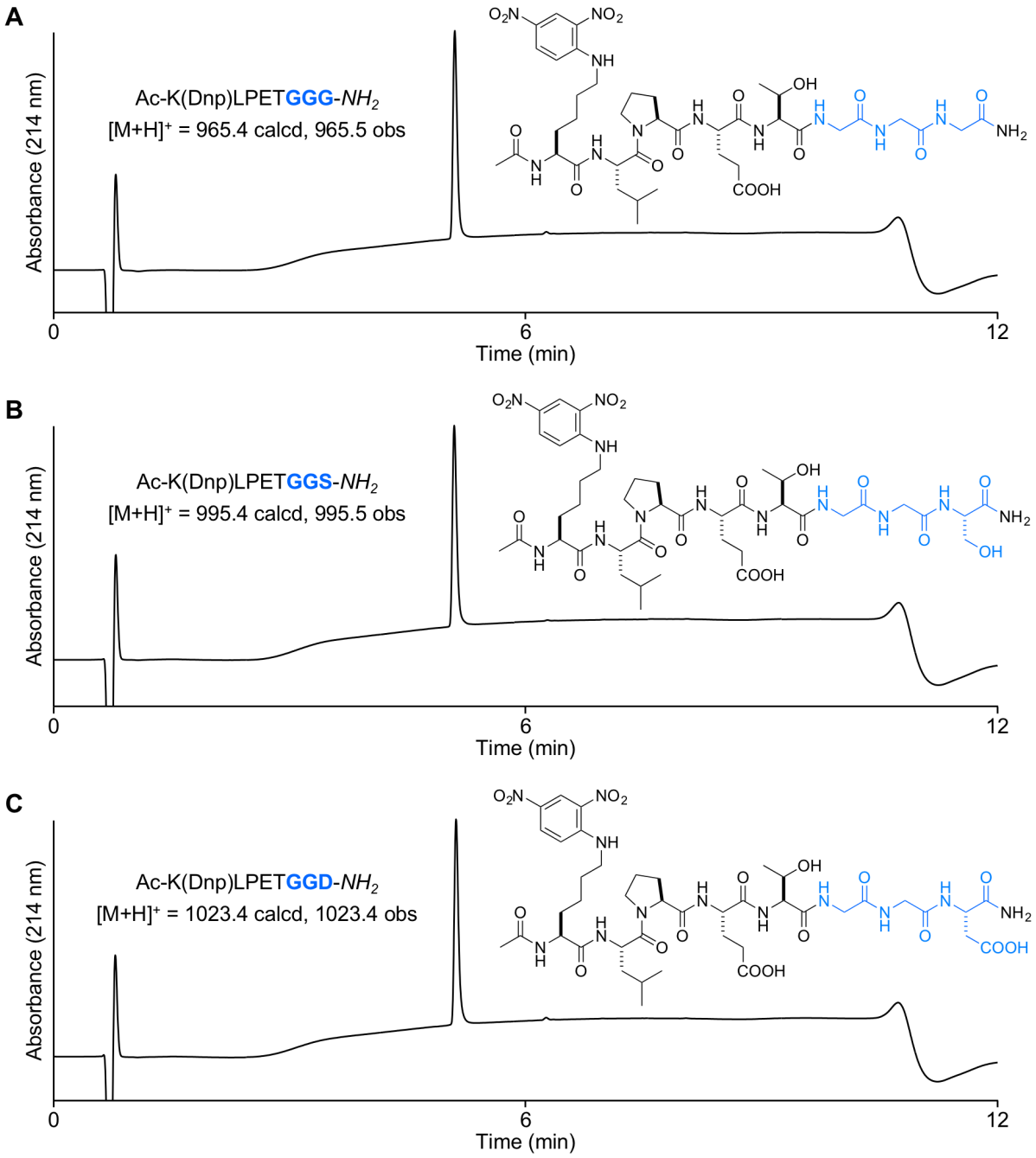


Figure S2. RP-HPLC and LC-ESI-MS characterization of purified (**A**) Ac-K(Dnp)LPETGGG-*NH*$_2$, (**B**) Ac-K(Dnp)LPETGGS-*NH*$_2$, and (**C**) Ac-K(Dnp)LPETGGD-*NH*$_2$. All calculated values for $[M+H]^+$ are monoisotopic molecular weights. RP-HPLC conditions for panels **A**-**C**: Phenomenex Kinetex® 2.6 µm C18 100 Å column (100 x 2.1 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic acid) mobile phase at 0.4 mL/min, method: hold 5% MeCN 0.0-1.0 min, linear gradient of 5-90% MeCN 1.0-7.0 min, hold 90% MeCN 7.0-9.0 min, linear gradient of 90-5% MeCN 9.0-9.1 min, re-equilibrate at 5% MeCN 9.1-12.0 min.

Table S1. Mass spectrometry (LC-ESI-MS) characterization of relevant reaction components from peptide model studies.[a]

Peptide		Mass (m/z)	
		calcd	obs
Substrates	Ac-K(Dnp)LPET**GGH**G-*OH*	1103.5	1103.5
	Ac-K(Dnp)LPET**GGH**-*OH*	1046.5	1046.4
	Ac-K(Dnp)LPET**GGH**-*NH_2*	1045.5	1045.5
	Ac-K(Dnp)LPET**GGG**-*NH_2*	965.4	965.4
	Ac-K(Dnp)LPET**GGS**-*NH_2*	995.4	995.4
	Ac-K(Dnp)LPET**GGD**-*NH_2*	1023.4	1023.4
Ligation Products	Ac-K(Dnp)LPET**G**-*OH*	852.4	852.3
	Ac-K(Dnp)LPET**G**-*NH_2*	851.4	851.4
	Ac-K(Dnp)LPET**GG**-*OH*	909.4	909.4
	Ac-K(Dnp)LPET**GG**-*NH_2*	908.4	908.4
	Ac-K(Dnp)LPET**GGG**-*OH*	966.4	966.4
	Ac-K(Dnp)LPET**GGG**-*NH_2*	965.4	965.5
Hydrolysis	Ac-K(Dnp)LPET-*OH*	795.4	795.3

[a]Calculated (calcd) and observed (obs) masses represent $[M+H]^+$ ions (monoisotopic). [Ac = acetyl capping group, Dnp = 2,4-dinitrophenyl chromophore, -*OH* = native carboxylic acid C-terminus, -*NH_2* = primary amide at C-terminus].

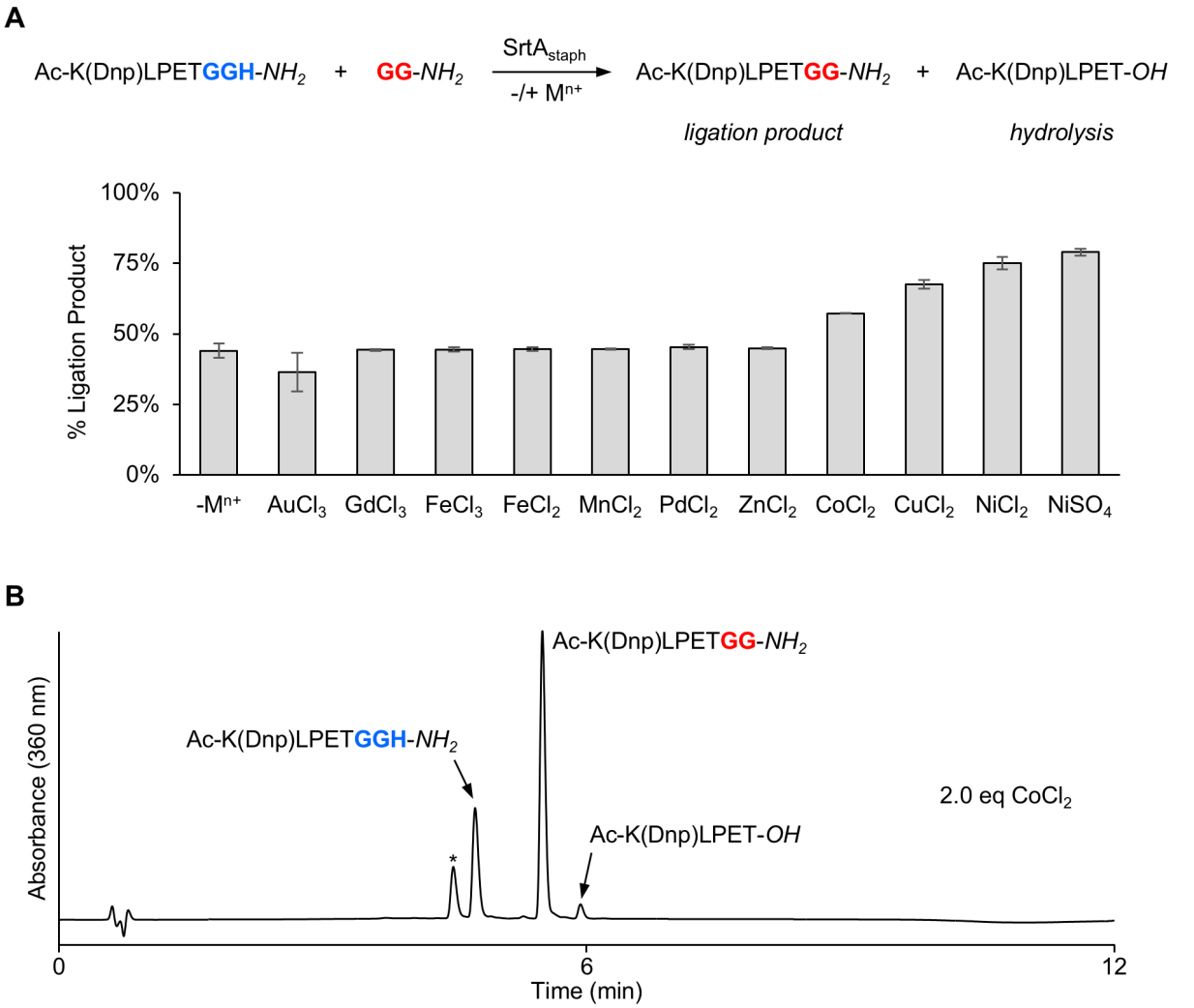
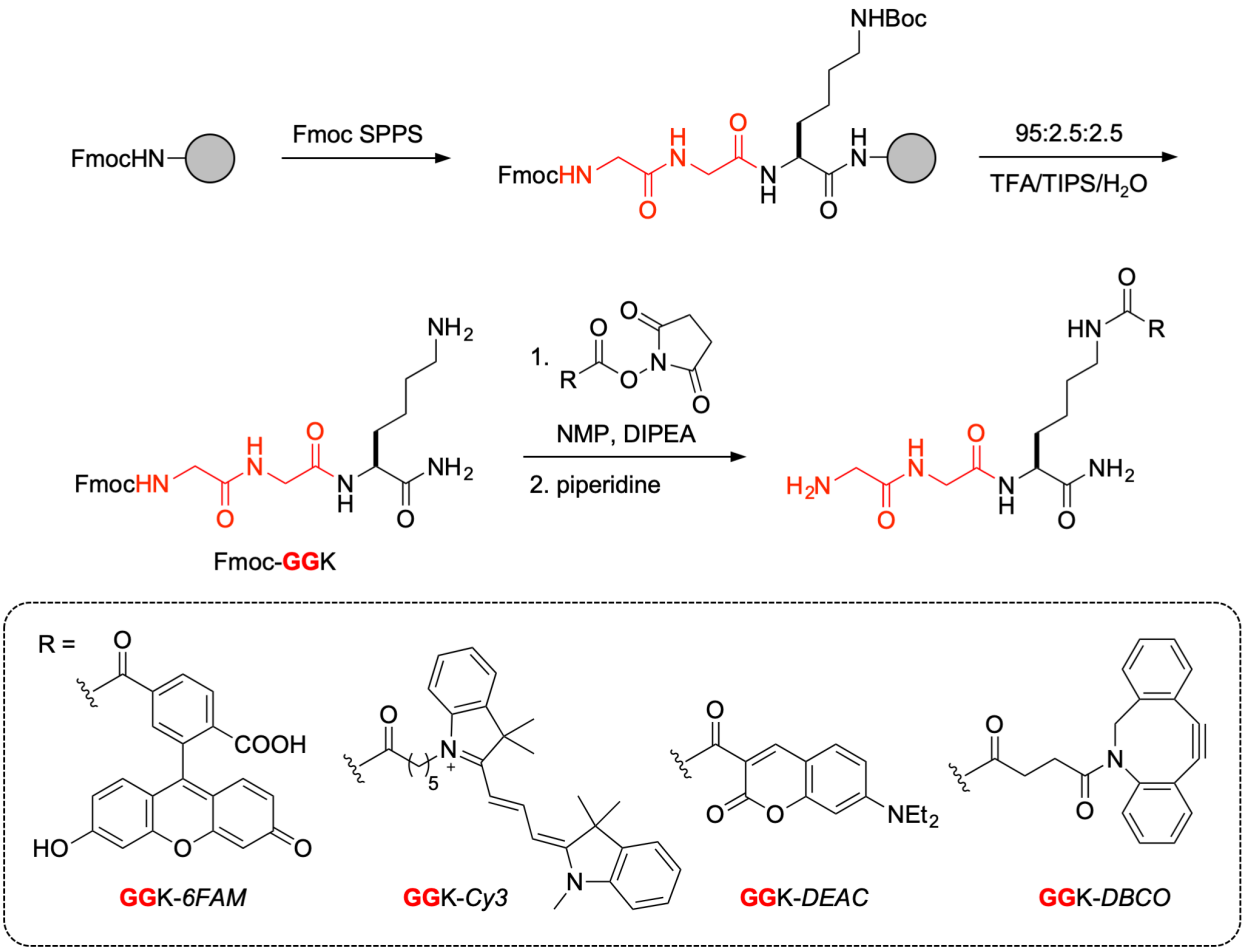


Figure S3. (**A**) Effect of different metal additives on MA-SML. Reaction conditions: 100 μM Ac-K(Dnp)LPETGGH-*NH*$_2$, 100 μM GG-*NH*$_2$, 10 μM SrtA$_{staph}$, and 0 / 200 μM metal salt (-*NH*$_2$ = primary amide at peptide C-terminus). Reactions were incubated at room temperature for 6 h and then analyzed by RP-HPLC. Estimates of reaction progress were obtained by comparing peak areas derived from the 360 nm RP-HPLC chromatogram. All reactions were performed in triplicate and values for % ligation product are reported as the mean ± standard deviation. In addition to the expected peaks for the substrate peptide (Ac-K(Dnp)LPETGGH-*NH*$_2$), ligation product (Ac-K(Dnp)LPETGG-*NH*$_2$), and hydrolysis product (Ac-K(Dnp)LPET-*OH*), some reactions displayed low levels of unidentified by-products in the 360 nm chromatogram. These peak areas are included in the overall estimate for % ligation product, and with the exception of reactions involving $CoCl_2$, these by-products never exceeded 4% of the total reaction mixture. (**B**) Representative RP-HPLC chromatogram for model ligation reaction utilizing $CoCl_2$ as the metal additive (same reaction conditions as in **A**). In addition to the expected components of the reaction mixture, a significant by-product (*) exhibiting the absorbance of the Dnp chromophore was observed. Based on peak area, this species represented ~14% of the reaction mixture. The identity of this species was not determined.

Synthesis and characterization of functionalized diglycine nucleophiles.

General. Functionalized nucleophiles were synthesized using a combination of manual Fmoc solid phase peptide synthesis (SPPS) followed by solution phase reactions for installing the desired modification (**Scheme S1** and **Scheme S2**). For SPPS, syntheses were performed in 15 mL polypropylene synthesis vessels fitted with appropriate frits and inlet/outlet caps. All manipulations (washing, coupling, deprotection) were conducted at room temperature and included gentle agitation on a bench-top rocking platform. All materials, including standard Fmoc amino acids, Fmoc Rink amide MBHA resin, and reagents for coupling, deprotection, and resin cleavage were obtained from commercial sources and used without further purification. A colorimetric ninhydrin test kit (Anaspec) was utilized to assess the success of SPPS coupling reactions. Incorporation of the desired label was achieved using the following commercially available activated esters: 6FAM-NHS (Anaspec), Cy3-NHS (Lumiprobe), DEAC-NHS (Sigma), DBCO-NHS (Conju-probe), TFP-dPEG®$_4$-(m-dPEG®$_{11}$)$_3$-ester (Quanta Biodesign).

Scheme S1. Synthesis of fluorescent and DBCO-modified nucleophiles (GGK-*6FAM*, GGK-*Cy3*, GGK-*DEAC*, and GGK-*DBCO)*.



Synthesis of Fmoc-GGK. A solid phase synthesis vessel was loaded with 455 mg (0.20 mmol) of Fmoc-protected Rink amide MBHA resin (0.44 mmol/g). The resin was first washed/swollen with NMP. Fmoc removal was achieved by treatment with 10 mL of 80:20 NMP/piperidine (2x, 10 min per treatment), followed by washing with ~10 mL of NMP (3x, 5 min per wash). Suitably protected amino acid building blocks were then coupled as follows: Fmoc-protected amino acid (0.60 mmol), HBTU (228 mg, 0.60 mmol), and DIPEA (174 µL, 1.0 mmol) were dissolved in 6 mL of NMP. This solution was mixed thoroughly, and then added to the deprotected resin. Couplings were incubated for ~1 h at room temperature. The resin was then washed with ~10 mL of NMP (3x, 5 min per wash) to remove unreacted components. Repeated cycles of Fmoc deprotection, coupling, and washing were then performed to assemble the desired peptide sequence. Cleavage from the solid support was achieved by first washing with ~10 mL of CH_2Cl_2 (3x, 5 min per wash), followed by treatment with 5 mL of 95:2.5:2.5 TFA/TIPS/H_2O (2x, 30 min per treatment). The combined cleavage solutions were concentrated on a rotary evaporator, and the remaining residue was then slowly added to 35 mL of dry ice-chilled diethyl ether. The precipitated peptide was collected by centrifugation and dried overnight under vacuum. The identity of the desired peptide (Fmoc-GGK) was confirmed by LC-ESI-MS ($[M+H]^+$ = 482.2 calcd, 482.3 obs). The crude material was solubilized in 0.5 mL of NMP (107 mg/mL final concentration) and used without further purification.

Synthesis of GGK-6FAM. Crude Fmoc-GGK (4.7 mg, 9.8 µmol) was combined with 0.5 molar equivalents of 6FAM-NHS and 3 molar equivalents of DIPEA in NMP (100 µL total reaction volume). The reaction was incubated at room temperature for 30 min and then treated with piperidine (25 µL). The reaction was incubated for an additional 30 min at room temperature to remove Fmoc, and then the GGK-*6FAM* product was purified directly from the reaction mixture by RP-HPLC (Phenomenex Luna 5 µm C18(2) 100 Å column (10 x 250 mm), aqueous (95% H_2O, 5% MeCN, 0.1% TFA) / MeCN (0.1% TFA) mobile phase at 4.0 mL/min, method: hold 20% MeCN 0.0-2.0 min, linear gradient of 20-70% MeCN 2.0-15.0 min, linear gradient of 70-90% MeCN 15.0-15.1 min, hold 90% MeCN 15.1-17.0 min, linear gradient of 90-20% MeCN 17.0-17.1 min, re-equilibrate at 20% MeCN 17.1-19.0 min). Pure fractions of GGK-*6FAM* were pooled and lyophilized, and the identity and purity of the product were confirmed by RP-HPLC and LC-ESI-MS (**Figure S4**). Prior to use in sortase-mediated ligation, GGK-*6FAM* was dissolved in 90:10 H_2O/DMSO and the concentration of the stock solution was estimated by diluting it into water (10-fold) and using the absorbance of fluorescein at 495 nm (extinction coefficient = 75,000 $M^{-1}cm^{-1}$).[1,2]

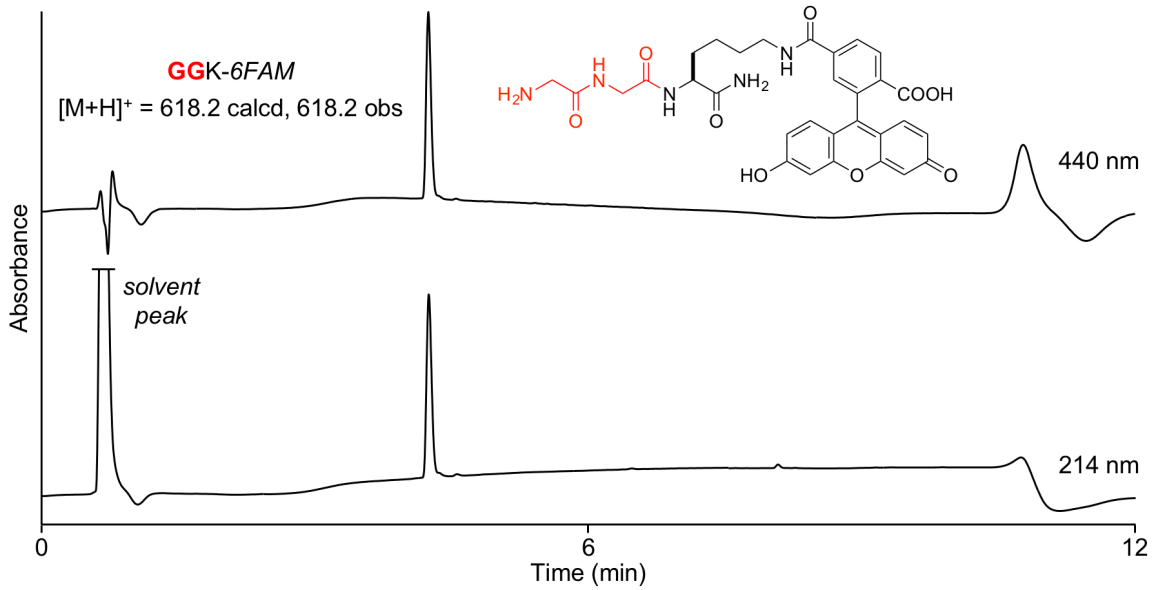


Figure S4. RP-HPLC and LC-ESI-MS characterization of purified GGK-*6FAM*. Calculated value for $[M+H]^+$ is monoisotopic molecular weight. RP-HPLC conditions: Phenomenex Kinetex® 2.6 µm C18 100 Å column (100 x 2.1 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic acid) mobile phase at 0.4 mL/min, method: hold 5% MeCN 0.0-1.0 min, linear gradient of 5-90% MeCN 1.0-7.0 min, hold 90% MeCN 7.0-9.0 min, linear gradient of 90-5% MeCN 9.0-9.1 min, re-equilibrate at 5% MeCN 9.1-12.0 min.

Synthesis of GGK-Cy3. Crude Fmoc-GGK (4.1 mg, 8.5 µmol) was combined with 0.5 molar equivalents of Cy3-NHS and 3 molar equivalents of DIPEA in NMP (100 µL total reaction volume). The reaction was incubated at room temperature for 60 min and then treated with piperidine (25 µL). The reaction was incubated for an additional 30 min at room temperature to remove Fmoc, and then the GGK-*Cy3* product was purified directly from the reaction mixture by RP-HPLC (Phenomenex Luna 5 µm C18(2) 100 Å column (10 x 250 mm), aqueous (95% H_2O, 5% MeCN, 0.1% TFA) / MeCN (0.1% TFA) mobile phase at 4.0 mL/min, method: hold 20% MeCN 0.0-2.0 min, linear gradient of 20-90% MeCN 2.0-15.0 min, hold 90% MeCN 15.0-17.0 min, linear gradient of 90-20% MeCN 17.0-17.1 min, re-equilibrate at 20% MeCN 17.1-19.0 min). Pure fractions of GGK-*Cy3* were pooled and lyophilized, and the identity and purity of the product were confirmed by RP-HPLC and LC-ESI-MS (**Figure S5**). Prior to use in sortase-mediated ligation, GGK-*Cy3* was dissolved in DMSO and the concentration of the stock solution was estimated by diluting it into water (100-fold) and using the absorbance of Cy3 at 555 nm (extinction coefficient = 150,000 $M^{-1}cm^{-1}$).[3,4]

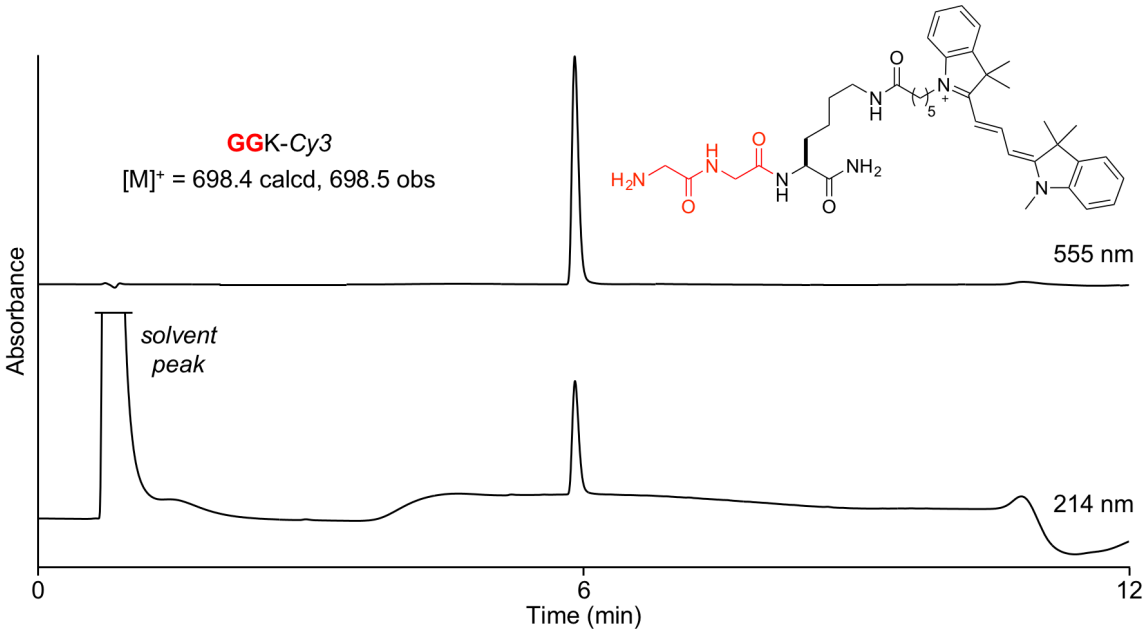


Figure S5. RP-HPLC and LC-ESI-MS characterization of purified GGK-*Cy3*. Calculated value for $[M]^+$ is monoisotopic molecular weight. RP-HPLC conditions: Phenomenex Kinetex® 2.6 µm C18 100 Å column (100 x 2.1 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic acid) mobile phase at 0.4 mL/min, method: hold 5% MeCN 0.0-1.0 min, linear gradient of 5-90% MeCN 1.0-7.0 min, hold 90% MeCN 7.0-9.0 min, linear gradient of 90-5% MeCN 9.0-9.1 min, re-equilibrate at 5% MeCN 9.1-12.0 min.

Synthesis of GGK-DEAC. Crude Fmoc-GGK (5.7 mg, 12 µmol) was combined with 0.5 molar equivalents of DEAC-NHS and 3 molar equivalents of DIPEA in NMP (100 µL total reaction volume). The reaction was incubated at room temperature for 90 min and then treated with piperidine (25 µL). The reaction was incubated for an additional 90 min at room temperature to remove Fmoc, and then the GGK-*DEAC* product was purified directly from the reaction mixture by RP-HPLC (Phenomenex Luna 5 µm C18(2) 100 Å column (10 x 250 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic) mobile phase at 4.0 mL/min, method: hold 10% MeCN 0.0-2.0 min, linear gradient of 10-90% MeCN 2.0-12.0 min, hold 90% MeCN 12.0-14.0 min, linear gradient of 90-10% MeCN 14.0-14.1 min, re-equilibrate at 10% MeCN 14.1-17.0 min). Pure fractions of GGK-*DEAC* were pooled and lyophilized, and the identity and purity of the product were confirmed by RP-HPLC and LC-ESI-MS (**Figure S6**). Two stock solutions of GGK-*DEAC* were prepared for use in sortase-mediated ligations: a more concentrated stock in 5:2 H_2O/DMSO and a more dilute stock in 97:3 H_2O/DMSO. The concentration of both stock solutions was estimated by diluting them into an excess of water (10 or 100-fold) and using the absorbance of DEAC at 429 nm (extinction coefficient = 46,800 $M^{-1}cm^{-1}$).[5,6]

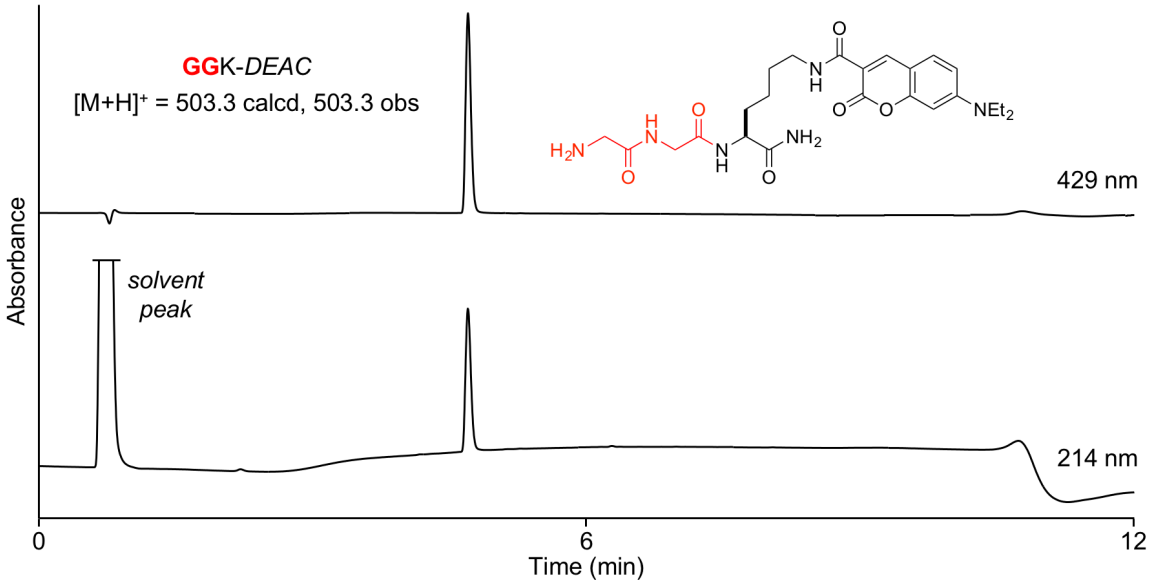


Figure S6. RP-HPLC and LC-ESI-MS characterization of purified GGK-*DEAC*. Calculated value for $[M+H]^+$ is monoisotopic molecular weight. RP-HPLC conditions: Phenomenex Kinetex® 2.6 µm C18 100 Å column (100 x 2.1 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic acid) mobile phase at 0.4 mL/min, method: hold 5% MeCN 0.0-1.0 min, linear gradient of 5-90% MeCN 1.0-7.0 min, hold 90% MeCN 7.0-9.0 min, linear gradient of 90-5% MeCN 9.0-9.1 min, re-equilibrate at 5% MeCN 9.1-12.0 min.

Synthesis of GGK-DBCO. Crude Fmoc-GGK (5.3 mg, 11 µmol) was combined with 0.5 molar equivalents of DBCO-NHS and 3 molar equivalents of DIPEA in NMP (100 µL total reaction volume). The reaction was incubated at room temperature for 25 min and then treated with piperidine (25 µL). The reaction was incubated for an additional 50 min at room temperature to remove Fmoc, and then the GGK-*DBCO* product was purified directly from the reaction mixture by RP-HPLC (Phenomenex Luna 5 µm C18(2) 100 Å column (10 x 250 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic) mobile phase at 4.0 mL/min, method: hold 10% MeCN 0.0-2.0 min, linear gradient of 10-90% MeCN 2.0-12.0 min, hold 90% MeCN 12.0-14.0 min, linear gradient of 90-10% MeCN 14.0-14.1 min, re-equilibrate at 10% MeCN 14.1-17.0 min). Pure fractions of GGK-*DBCO* were pooled and lyophilized, and the identity and purity of the product were confirmed by RP-HPLC and LC-ESI-MS (**Figure S7**). Prior to use in sortase-mediated ligation, GGK-*DBCO* was dissolved in 5:1 H_2O/DMSO and the concentration of the stock solution was estimated by diluting it into water (10-fold) and using the absorbance of DBCO at 309 nm (extinction coefficient = 12,000 $M^{-1}cm^{-1}$).[7,8]

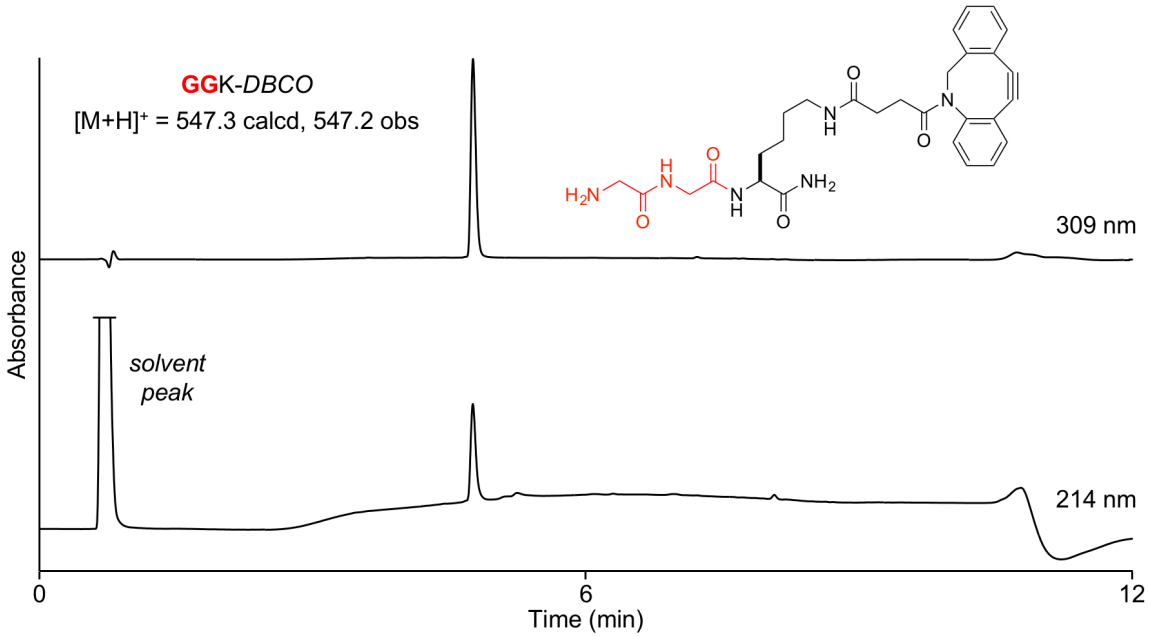
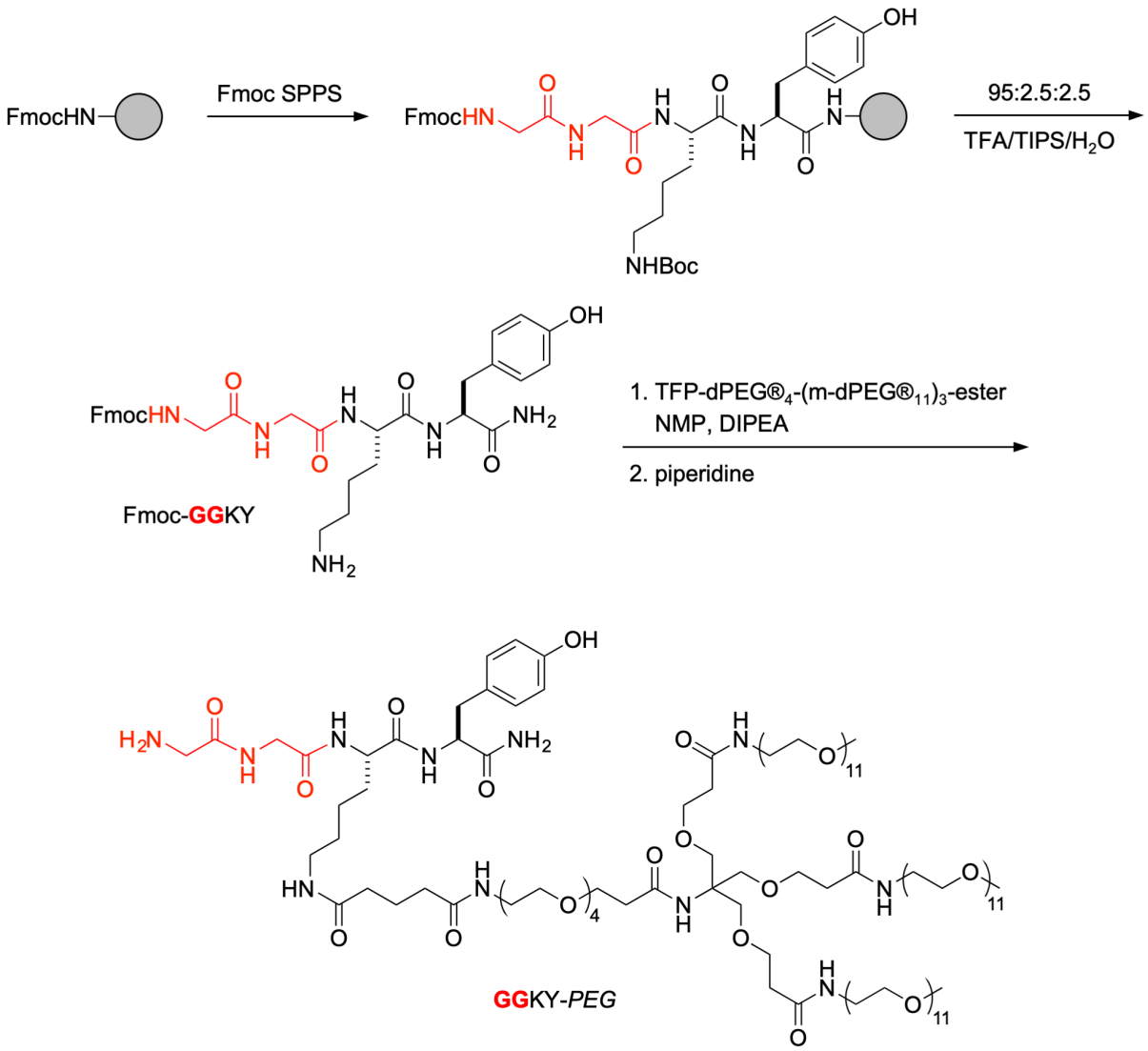


Figure S7. RP-HPLC and LC-ESI-MS characterization of purified GGK-*DBCO*. Calculated value for $[M+H]^+$ is monoisotopic molecular weight. RP-HPLC conditions: Phenomenex Kinetex® 2.6 µm C18 100 Å column (100 x 2.1 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic acid) mobile phase at 0.4 mL/min, method: hold 5% MeCN 0.0-1.0 min, linear gradient of 5-90% MeCN 1.0-7.0 min, hold 90% MeCN 7.0-9.0 min, linear gradient of 90-5% MeCN 9.0-9.1 min, re-equilibrate at 5% MeCN 9.1-12.0 min.

Scheme S2. Synthesis of PEG-modified nucleophile (GGKY-*PEG*).



Synthesis of Fmoc-GGKY. A solid phase synthesis vessel was loaded with 455 mg (0.20 mmol) of Fmoc-protected Rink amide MBHA resin (0.44 mmol/g). The resin was first washed/swollen with NMP. Fmoc removal was achieved by treatment with 10 mL of 80:20 NMP/piperidine (2x, 10 min per treatment), followed by washing with ~10 mL of NMP (3x, 5 min per wash). Suitably protected amino acid building blocks were then coupled as follows: Fmoc-protected amino acid (0.60 mmol), HBTU (228 mg, 0.60 mmol), and DIPEA (174 µL, 1.0 mmol) were dissolved in 6 mL of NMP. This solution was mixed thoroughly, and then added to the deprotected resin. Couplings were incubated for ~1 h at room temperature. The resin was then washed with ~10 mL of NMP (3x, 5 min per wash) to remove unreacted components. Repeated cycles of Fmoc deprotection, coupling, and washing were then performed to assemble the desired peptide sequence. Cleavage from the solid support was achieved by first washing with ~10 mL of CH_2Cl_2 (3x, 5 min per wash), followed by treatment with 5 mL of 95:2.5:2.5 TFA/TIPS/H_2O (2x, 30 min per treatment). The combined cleavage solutions were concentrated on a rotary evaporator, and the remaining residue was then slowly added to 35 mL of dry ice-chilled diethyl ether. The precipitated peptide was collected by centrifugation and dried overnight under vacuum. The identity of the desired peptide (Fmoc-GGKY) was confirmed by LC-ESI-MS ($[M+H]^+$ = 645.3 calcd, 645.4 obs). The crude material was solubilized in 0.5 mL of NMP (124 mg/mL final concentration) and used without further purification.

Synthesis of GGKY-PEG. Crude Fmoc-GGKY (6.9 mg, 11 µmol) was combined with 0.5 molar equivalents of TFP-dPEG®$_4$-(m-dPEG®$_{11}$)$_3$-ester and 3 molar equivalents of DIPEA in NMP (100 µL total reaction volume). The reaction was incubated at room temperature for 90 min and then treated with piperidine (25 µL). The reaction was incubated for an additional 60 min at room temperature to remove Fmoc, and then the GGKY-*PEG* product was purified directly from the reaction mixture by RP-HPLC (Phenomenex Luna 5 µm C18(2) 100 Å column (10 x 250 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic) mobile phase at 4.0 mL/min, method: hold 10% MeCN 0.0-2.0 min, linear gradient of 10-90% MeCN 2.0-12.0 min, hold 90% MeCN 12.0-14.0 min, linear gradient of 90-10% MeCN 14.0-14.1 min, re-equilibrate at 10% MeCN 14.1-17.0 min). Pure fractions of GGK-*PEG* were pooled and lyophilized, and the identity and purity of the product were confirmed by RP-HPLC and LC-ESI-MS (**Figure S8**). Prior to use in sortase-mediated ligation, GGKY-*PEG* was dissolved in H_2O and the concentration of the stock solution was estimated by using the absorbance of the single tyrosine residue at 280 nm (extinction coefficient = 1280 $M^{-1}cm^{-1}$).[9-11]

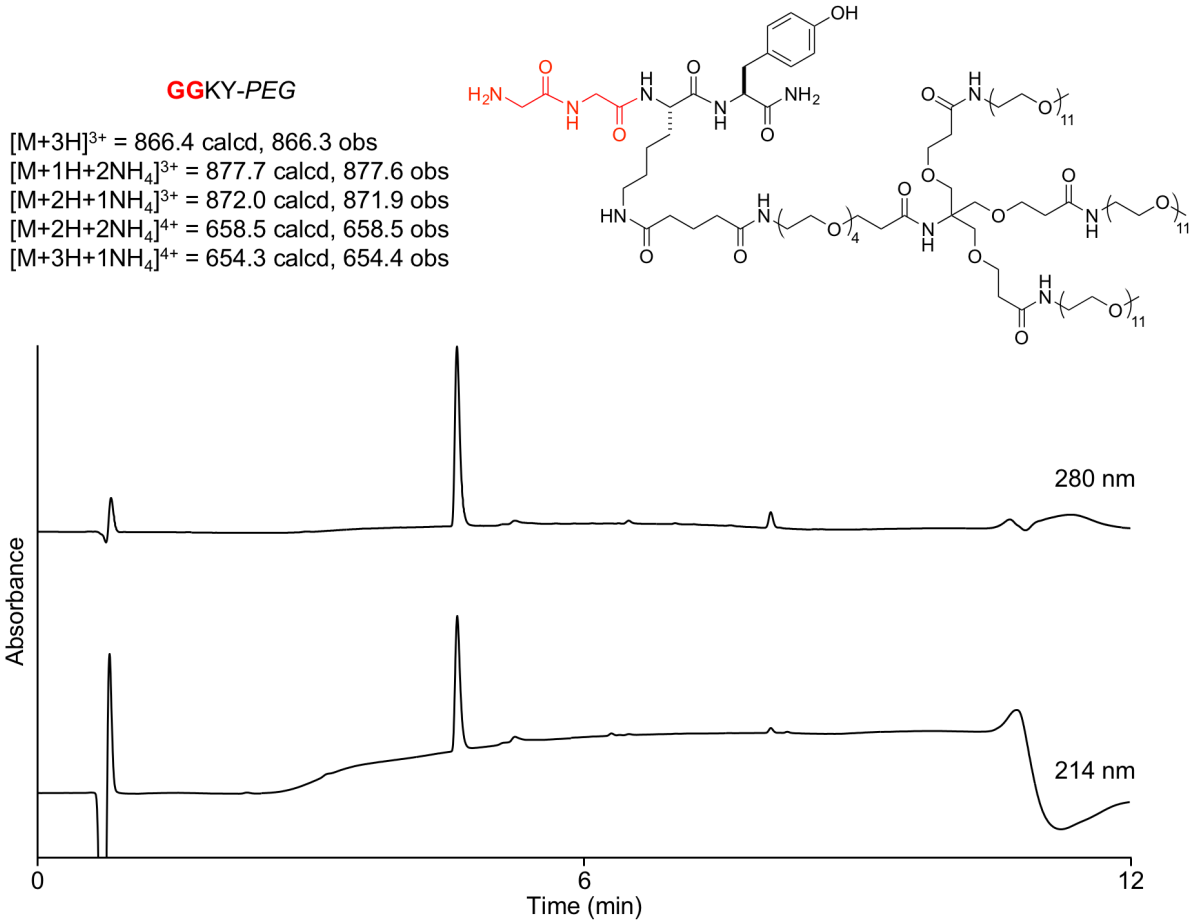


Figure S8. RP-HPLC and LC-ESI-MS characterization of purified GGKY-*PEG*. All calculated values for mass spectrometry are average molecular weight. RP-HPLC conditions: Phenomenex Kinetex® 2.6 µm C18 100 Å column (100 x 2.1 mm), aqueous (95% H_2O, 5% MeCN, 0.1% formic acid) / MeCN (0.1% formic acid) mobile phase at 0.4 mL/min, method: hold 5% MeCN 0.0-1.0 min, linear gradient of 5-90% MeCN 1.0-7.0 min, hold 90% MeCN 7.0-9.0 min, linear gradient of 90-5% MeCN 9.0-9.1 min, re-equilibrate at 5% MeCN 9.1-12.0 min.

Figure S9. Full sequences of proteins used in this study.

Staphylococcus aureus Δ59-sortase A ($SrtA_{staph}$)

MRGSSHHHHHHSSGLVPRGSHMQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATPEQLNRGVSFAEE
NESLDDQNISIAGHTFIDRPNYQFTNLKAAKKGSMVYFKVGNETRKYKMTSIRDVKPTDVGVLDEQKGKD
KQLTLITCDDYNEKTGVWEKRKIFVATEVK

DARP-LPETGGHH_5

MRGDLGKKLLEAARAGQDDEVRILMANGADVNAKDEYGLTPLYLATAHGHLEIVEVLLKNGADVNAVDAI
GFTPLHLAAFIGHLEIAEVLLKHGADVNAQDKFGKTAFDISIGNGNEDLAEILQKLNGGGGSGGGGSLPET
GGHHHHHH

Fyn-LPETGGHH_5

MRGSGVTLFVALYDYQADRWTDLSFHKGEKFQILDASPPGDWWEARSLTTGETGYIPSNYVAPVDSIQG
EQKLISEEDLGGGGSGGGGSLPETGGHHHHHH

FH8-LPETGGHH_5 (*initiator methionine absent in purified protein*)

PSVQEVEKLLHVLDRNGDGKVSAEELKAFADDSKCPLDSNKIKAFIKEHDKNKDGKLDLKELVSILSSGTS
ENLYFQGGGGGSGGGGSLPETGGHHHHHH

Aff-LPETGGHH_5 (*initiator methionine absent in purified protein*)

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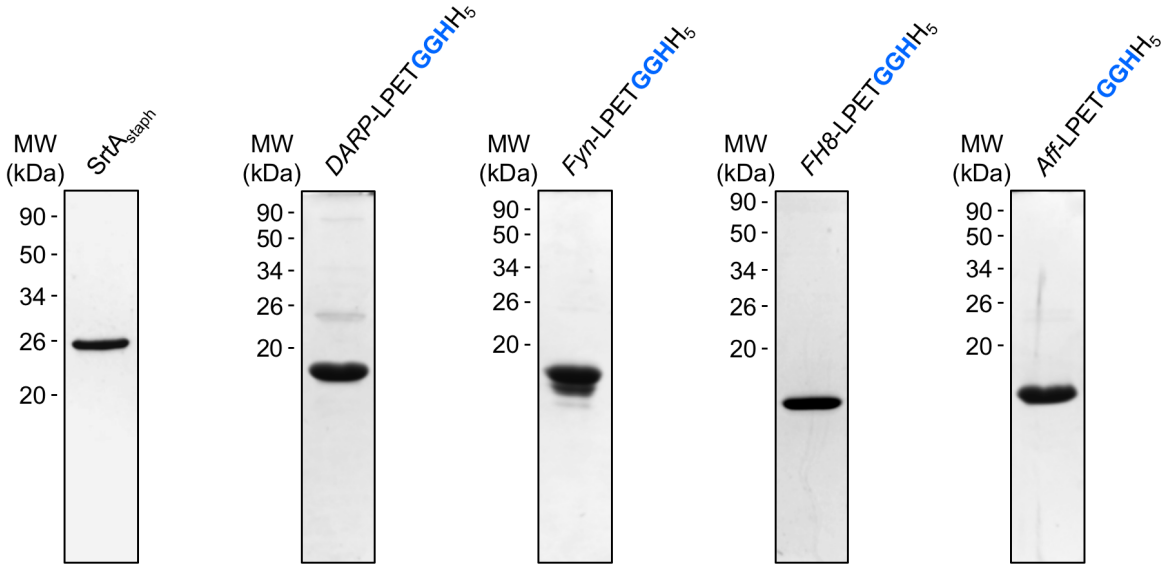

Figure S10. Reducing SDS-PAGE characterization of purified $SrtA_{staph}$, *DARP*-LPETGGHH_5, *Fyn*-LPETGGHH_5, *FH8*-LPETGGHH_5, and *Aff*-LPETGGHH_5.

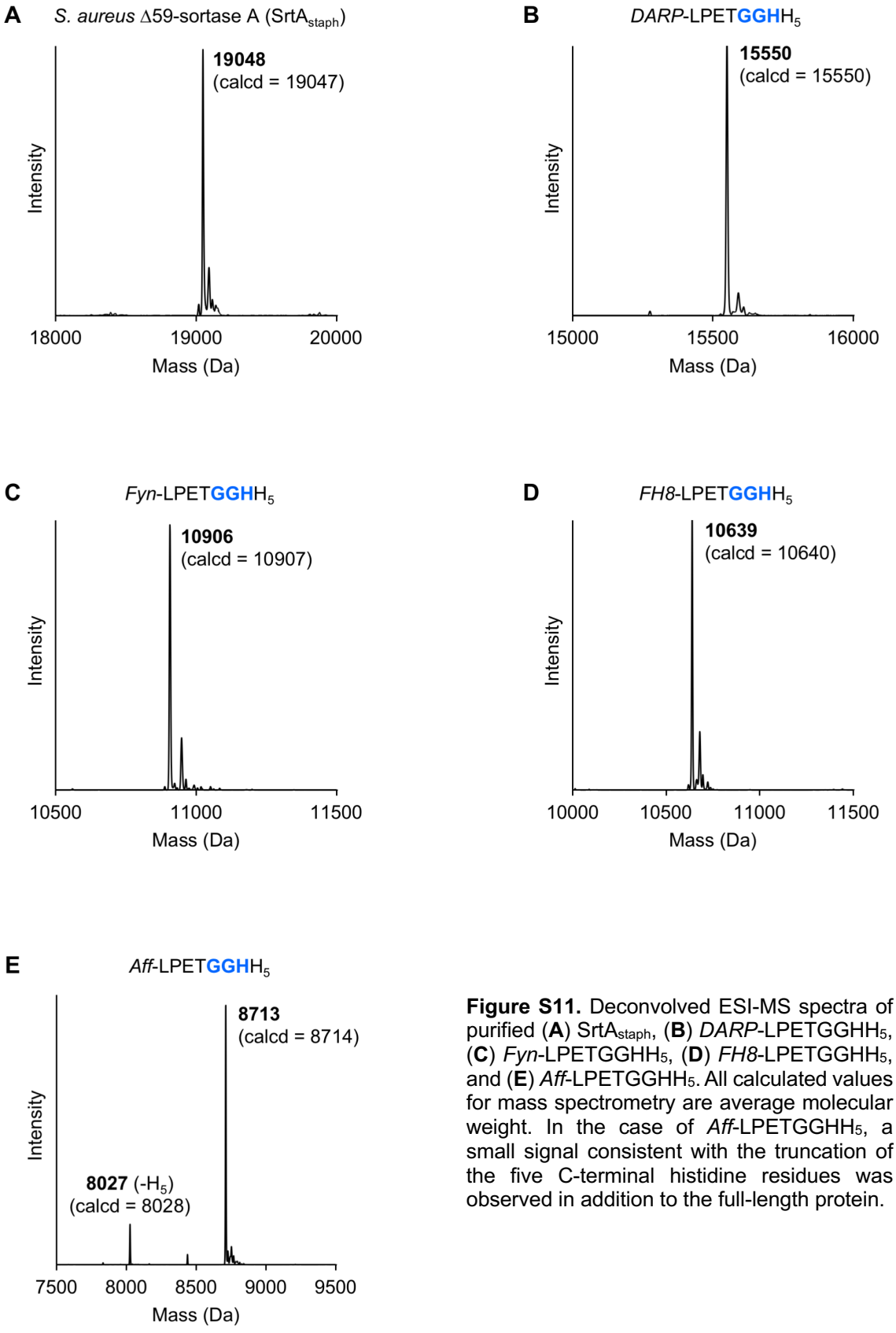

Figure S11. Deconvolved ESI-MS spectra of purified (**A**) $SrtA_{staph}$, (**B**) *DARP*-LPETGGHH_5, (**C**) *Fyn*-LPETGGHH_5, (**D**) *FH8*-LPETGGHH_5, and (**E**) *Aff*-LPETGGHH_5. All calculated values for mass spectrometry are average molecular weight. In the case of *Aff*-LPETGGHH_5, a small signal consistent with the truncation of the five C-terminal histidine residues was observed in addition to the full-length protein.

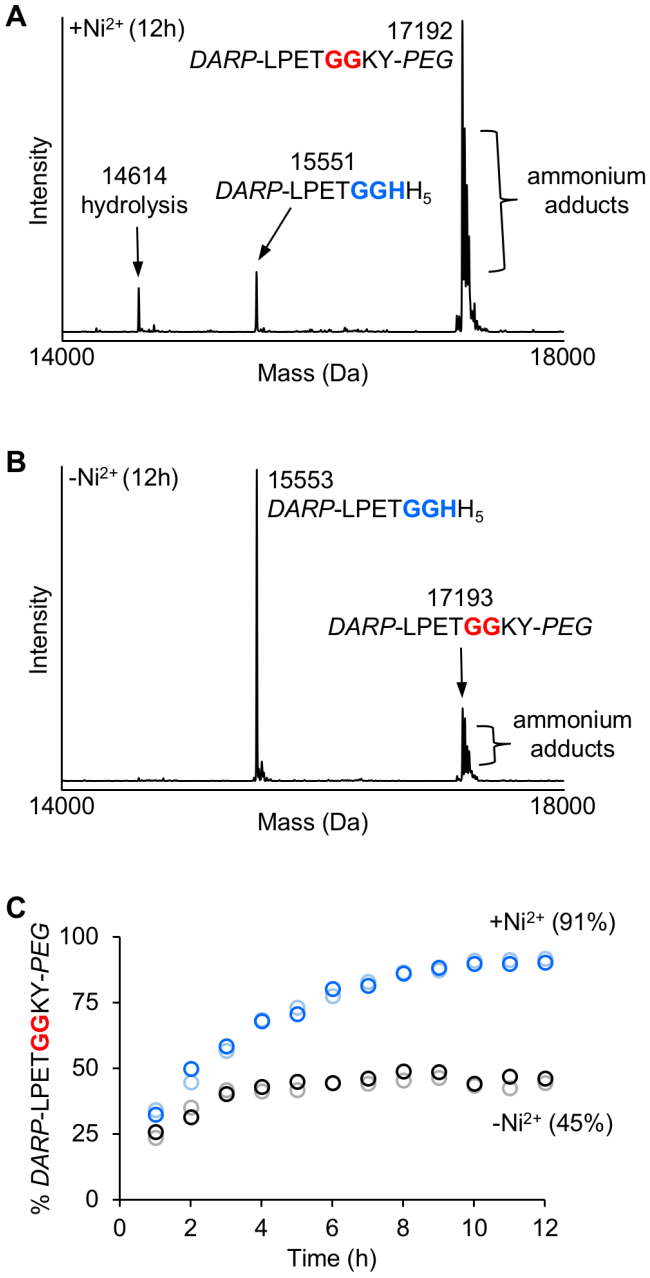

Figure S12. Representative ESI-MS spectra of sortase-mediated ligations utilizing 50 μM *DARP*-LPETGGHH$_5$, 50 μM GGKY-*PEG*, and 10 μM SrtA$_{staph}$ in the (**A**) presence or (**B**) absence of 200 μM $NiSO_4$ (12 h at room temperature). Calculated average molecular weight for *DARP*-LPETGGHH$_5$ = 15550 Da, *DARP*-LPETGGKY-*PEG* = 17191 Da, and hydrolysis = 14613 Da. (**C**) Time course demonstrating increased formation of the PEG-modified ligation product in the presence of Ni^{2+}. For this system, % product formed was estimated using LC-ESI-MS by comparing deconvoluted peak areas for unreacted *DARP*-LPETGGHH$_5$, hydrolysis, and the desired PEG-modified ligation product. Peak areas for the observed ammonium adducts were included as part of the total product formed. Blue/light blue circles represent two independent reactions containing Ni^{2+}, and black/grey circles represent two trials in the absence of Ni^{2+}. Values in parentheses represent the average % product formation for the two independent trials at the final timepoint.

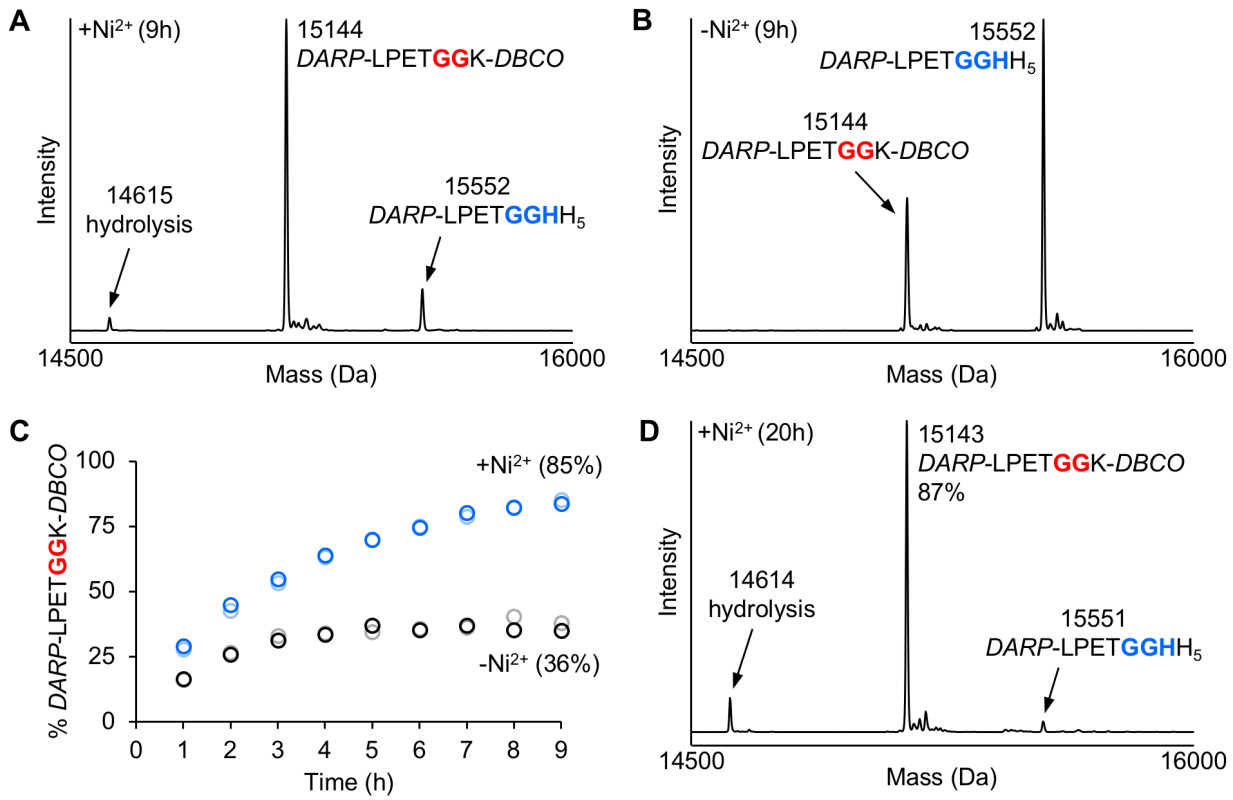

Figure S13. Representative ESI-MS spectra of sortase-mediated ligations utilizing 50 μM *DARP*-LPETGGHH$_5$, 50 μM GGK-*DBCO*, and 10 μM SrtA$_{staph}$ in the (**A**) presence or (**B**) absence of 200 μM $NiSO_4$ (9 h at room temperature). Calculated average molecular weight for *DARP*-LPETGGHH$_5$ = 15550 Da, *DARP*-LPETGGK-*DBCO* = 15142 Da, and hydrolysis = 14613 Da. (**C**) Time course demonstrating increased formation of the DBCO-modified ligation product in the presence of Ni^{2+}. For this reaction, % product formed was estimated using LC-ESI-MS by comparing deconvoluted peak areas for unreacted *DARP*-LPETGGHH$_5$, hydrolysis, and the desired DBCO-modified ligation product. Blue/light blue circles represent two independent reactions containing Ni^{2+}, and black/grey circles represent two trials in the absence of Ni^{2+}. Values in parentheses represent the average % product formation for the two independent trials at the final timepoint. (**D**) Deconvoluted ESI-MS spectrum of MA-SML (+Ni^{2+}) reaction after a 20 h incubation at room temperature (reagent concentrations identical to panel **A**). A minor increase in the extent of hydrolysis was observed as compared to the 9 h reaction, however the extent of product formation remained high (87% as estimated by LC-ESI-MS). This material was used without further purification for a subsequent strain-promoted azide-alkyne cycloaddition (see main text **Figure 5C**).

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