## Intrinsic Bioconjugation for Site-Specific Protein PEGylation

## at N-Terminal Serine

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# **Supporting Information**

### A. Synthesis of PEG-Salicylaldehyde and Ligation Reaction Conditions

Ribonuclease A ( $\geq$ 60% (SDS-PAGE), and subtilisin were purchased from Sigma Aldrich. Starting PEG material (t-boc-N-amido-sPEG<sub>12</sub>-acid, >94%) was purchased from Quanta Biodesign. Other reagents were obtained from commercial sources and used without additional purification.

HPLC analysis and purifications were performed on a Agilent instrument. For analytical analysis, a  $C_{18}$  reversed-phase HPLC column was used (Peeke Scientific). Samples were eluted with a 5-95% acetonitrile/water gradient (0.1% TFA) in 20 minutes with a flow rate of 0.7 mL/min and monitored at 214 nm. For purifications, semi-preparative  $C_{18}$  reversed-phase HPLC columns were used (Peeke Scientific). Samples were eluted with a 5-95% acetonitrile/water gradient (0.1% TFA) in 50 minutes with a flow rate of 2.5 mL/min, and monitored at 214 nm.

All mass spectrometry data was obtained on either a Agilent 1100 Series LCMSD VL Mass Spectrometer or a Bruker UltrafleXtreme MALDI-TOF mass spectrometer in positive-ion mode. Matrix: Sinapic acid in acetonitrile with 0.01% TFA.

*Preparation of S-Protein:* 40  $\mu$ L of 1% subtilisin in 100 mM Tris pH 8.0 was added dropwise to a stirring solution of 5 mL of 2% RNase A (100 mM Tris pH 8.0) on ice. After 12-16 h, the reaction was quenched with 2 M HCl to pH < 4 and immediately purified by HPLC. The fractions containing the S-protein and the S-peptide were identified by LCMS.<sup>1</sup>

General Procedure for the Preparation of PEG-Salicylaldehyde: ~25 mg of t-boc-N-amido-sPEG<sub>12</sub>-acid was suspended in dry DCM and 1.2 eq. of DIC, 1.1 eq. of salicylaldehyde, and 0.1 eq. of DMAP was added. The reaction was stirred at RT for 16 hours and then purified by reversed-phase HPLC.

<sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O):  $\delta = 10.05$  (s, 1H), 8.01 (d, J = 7.8 Hz, 1 H), 7.83 (t, J = 7.8 Hz, 1 H), 7.58 (t, J = 7.8 Hz, 1 H), 7.34 (d, J = 7.8 Hz, 1 H), 3.98 (t, J = 6 Hz, 2 H), 3.77-3.61 (m, 44 H), 3.60 (t, J = 5.4 Hz, 2 H), 3.27 (t, J = 5.4 Hz, 2 H), 3.07 (t, J = 6 Hz, 2 H), 1.44 (s, 9 H).

General Procedure for Ligation: S-protein (2 mg) or PTH 1-34 (2 mg) were dissolved in pyridine/acetic acid (1:1 v/v) to a final concentration of ~10 mM and PEG-salicylaldehyde (~2 equiv.) was added. The reaction was stirred at room temperature and monitored using MALDI-TOF and HPLC. Following completion of the reaction (consumption of salicylaldehyde to form the acetal intermediate), the solvent was removed by lyophilization and the intermediate product was treated with TFA/H<sub>2</sub>O/*i*-Pr<sub>3</sub>SiH (94/5/1, v/v/v) for 2 hr to give the product containing a native amide bond at the ligation site.

*Circular Dichroism (CD) Spectroscopy*: Circular Dichroism spectra were measured on an Aviv spectrophotometer (Lakewood, NJ). Spectra were acquired at concentrations of 10  $\mu$ M oligomer in 10 mM PBS (pH 7.5) in a 1 mm cuvette. Wavelength-dependent spectra were acquired from 260 nm to 180 nm (data pitch 0.5 nm, scan speed 50 nm/min, 4 sec, 1 nm bandwidth and 10 accumulations). Mean residue ellipticity values were calculated from the equation MRE = ( $\Theta$ sample –  $\Theta$ buffer)/(L • c • n ), where  $\Theta$  is observed signal in millidegrees, L is the length of the cuvette in cm, c is the concentration of peptide in dmol/cm<sup>3</sup>, and n is the number of residues in the peptide oligomers.

### C. Analytical HPLC data



**Supplementary Figure 1.** Analytical HPLC analysis of purified cleaved PEG PTH (1-34).

#### C. Proposed mechanism of Ser/Thr ligation



Supplementary Figure 2. Proposed ligation mechanism to form native Ser/Thr linkages.

### References

1) A. T. Fafarman and S. G. Boxer, J. Phys. Chem. B, 2010, 114, 13, 536.