Methods

Stability Study

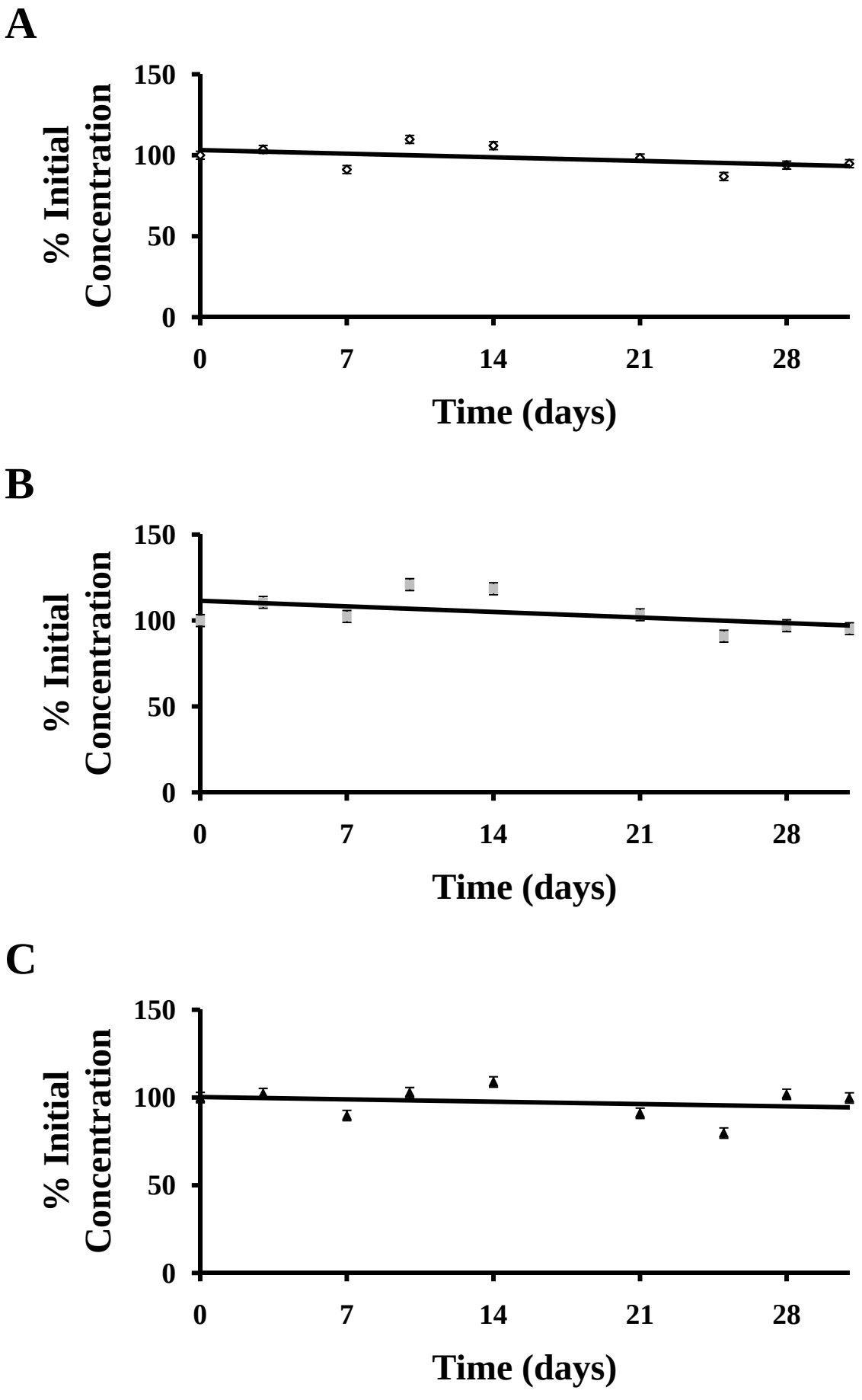
Individual (0.3 and 1.0 mg/mL) and combination solutions of DNSP-5, DNSP-11, and DNSP-17 were made in sterile citrate buffer (10 mM Citrate + 150 mMNaCl, pH 5.0). Samples were then stored at -80 °C and 37 °C for 0, 3, 7 10, 14, 17, 21, 25, 28, or 31 days. At these time points, aliquots were analyzed for degradation using RP-HPLC (Waters Breeze System) with dH_20 (HPLC grade) + 0.1% trifluoroacetic acid (TFA) as the aqueous mobile phase. Samples were loaded to a C4 column (4.6 mm x 75 mm, 300 Å pore size, GRACE/Vydac 214TP54, Deerfield, IL) at a flow rate of 1 mL/min and the column flow through was monitored at 214 nm with a Waters 2486 dual-wavelength UV/VIS detector. Samples were eluted with a linear gradient of the organic mobile phase (acetonitrile + 0.1% TFA), to a final aqueous:organic phase ratio of 75:25 after 30 minutes. All solvents were HPLC grade, degassed and filtered prior to use. At 31 days, aliquots were subjected to LC-MS analysis.

Far-UV circular dichroism spectroscopy

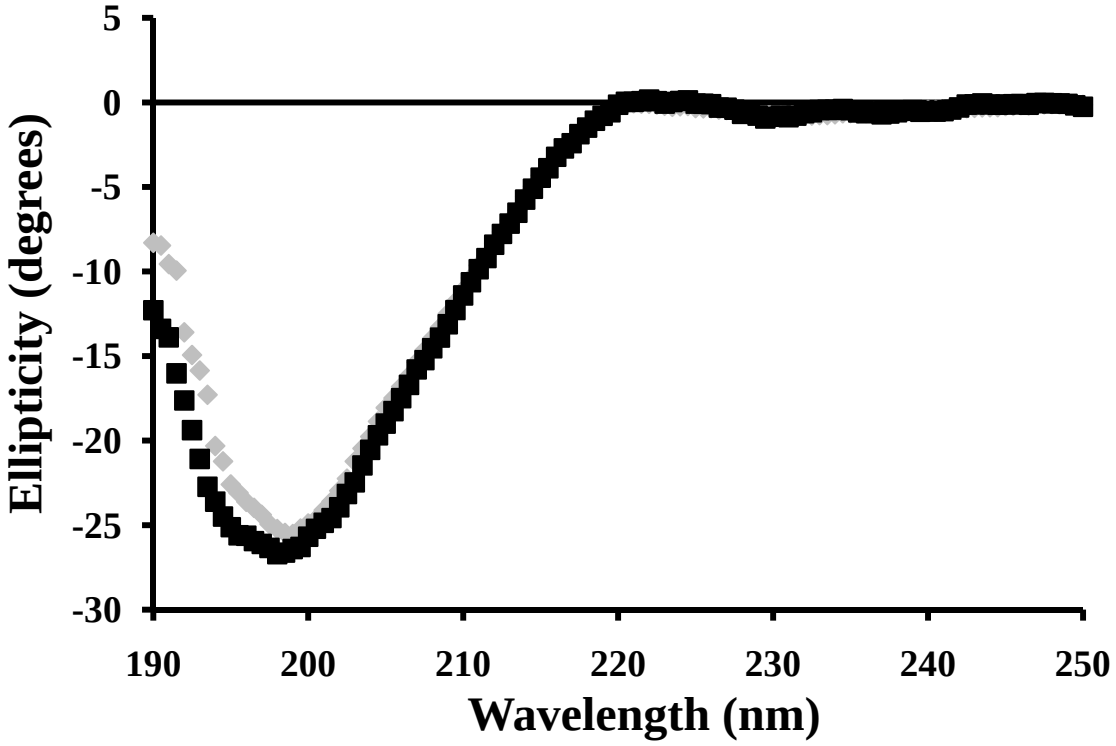
CD measurements were performed for a combined purified peptide sample (DNSP-5, 130 μM; DNSP-11, 21 μM; DNSP-17, 13 μM) in 50 mM sodium phosphate buffer, pH 7.0. Measurements were made in a 1 mm quartz cuvette using a Jasco J-810 spectrophotometer. Spectra were recorded as the average of four far-UV wavelength scans from 250 to 190 nm with 0.5 nm steps and 8 second averaging time. A calculated spectra was obtained from the individual spectra shown in Figure 1.

Supplemental Figure 1: The DNSPs are intrinsically stable *in vitro*.. **(A)** DNSP-5, **(B)** DNSP-11, and **(C)** DNSP-17 were stored in citrate buffer, pH 5.0 at either 37°C or -80°C (data not shown) for 31 days. These samples were then run on RP-HPLC to look for degradation of the peptides. The peak corrected peak height was used to determine changes in peptide concentration over time. Error bars indicate SEM.

Supplemental Figure 2: The DNSPs do not interact *in vitro*. The calculated additive spectra of the individual peptides overlays with the measured tripeptide mixture spectrum, indicating that the three peptides do not interact *in vitro*.



Supplemental Figure 1 (Kelps *et al.*)



Supplemental Figure 2 (Kelps *et al.*)