Supplementary information to "Identification of Novel Small Molecules that Bind to Two Different Sites on the Surface of Tetanus Toxin C Fragment" by Monique Cosman, Felice C. Lightstone, V. V. Krishnan, Loreen Zeller, Maria C. Prieto, Diana C. Roe and Rod Balhorn (page 1 of 3).

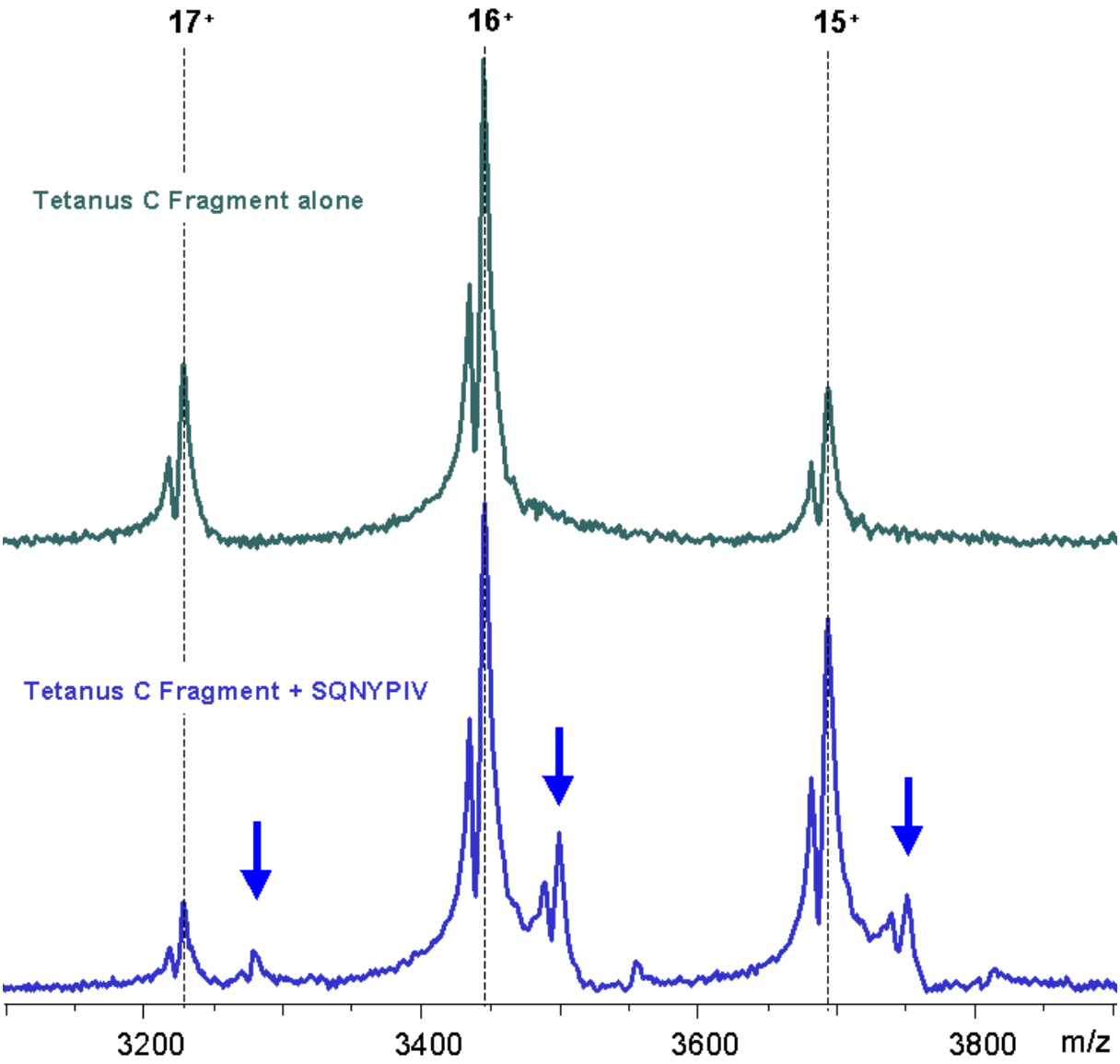

Figure S1. ESI-MS spectra of TetC and the TetC/SQNYPIV complex: Binding of the peptide to TetC is confirmed by the presence of shifted peaks, indicated by the blue arrows, relative to the corresponding peaks of TetC alone. The changes in the abundance of the 15^+ and 17^+ charge state distributions also imply that a change in the conformation of the protein may have occurred upon ligand binding.

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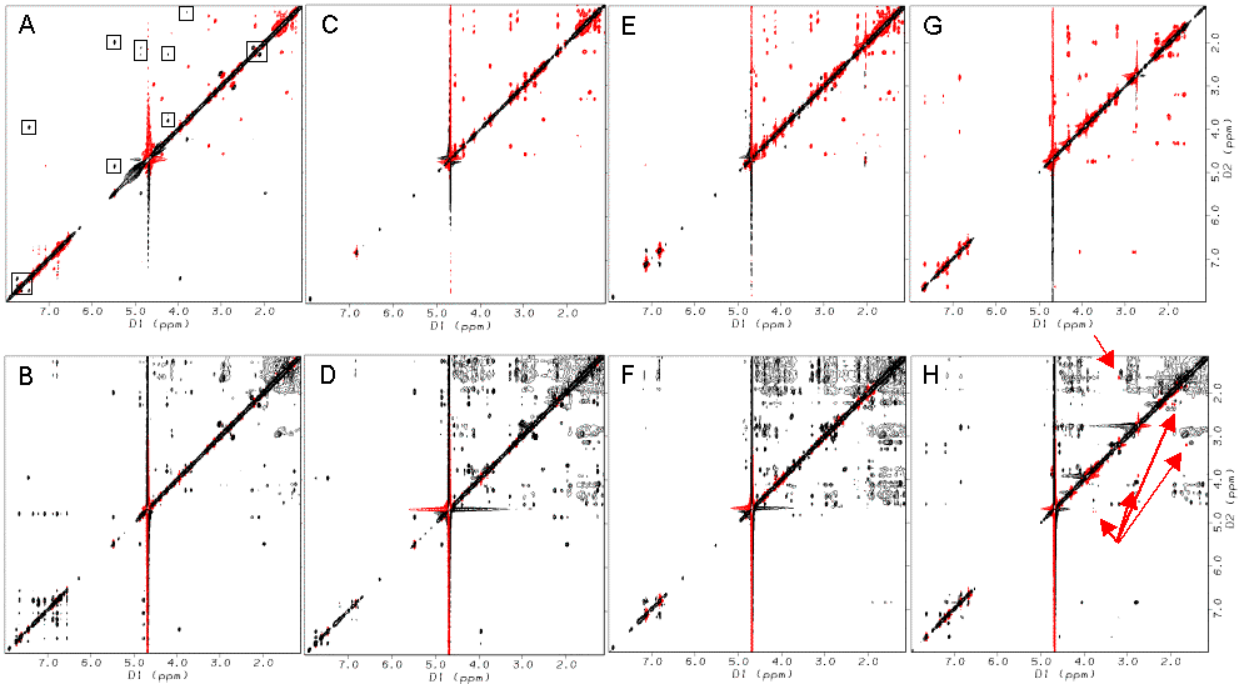

Figure S2. Top panels: 900 ms NOESY spectra of ligand mixtures in D_2O at 30 ^oC; bottom panels: 300 ms trNOESY spectra of ligand mixtures plus TetC in D_2O at 30 ^oC. The concentrations of ligands and TetC, as well as the TetC:ligand ratios, are given below. The top panels of the 900 ms spectra are plotted at 2 times lower level than the bottom panels of the 300 ms spectra for presentation purposes. Negative crosspeaks (relative to the diagonal) are in red, while positive crosspeaks are in black. The ligand mixtures are composed of: (A,B) doxorubicin, lavendustin A, and MP-biocytin; (C) MP-biocytin and (D) doxorubicin and MP-biocytin; (E,F) MP-biocytin and SQNYPIV; and (G,H) Sar-RGDSP and Try-Glu-Trp (the red arrows denote the negative crosspeaks belonging to Sar-RGDSP). Note that doxorubicin shows several crosspeaks that are positive even in the absence of TetC (boxed cross peaks in A). Concentrations of TetC and ligands used in these experiments are given below.

Ligand	[Ligand] (μM)	[TetC] (μM)	[TetC]:[Ligand]	binding
Doxorubicin	1041.3	52.0	1:20	yes
MP-biocytin	1149.3	52.0	1:22	no
Lavendustin	1154.2	52.0	1:22	yes
MP-biocytin	1232.5	71.9	1:17	yes
Doxorubicin	1048.6	67.5	1:16	yes
SQNYPIV	1180.6	68.4	1:17	yes
MP-biocytin	1173.9	68.4	1:17	yes
Sar-RGDSP	1114.1	64.8	1:17	no
Tyr-Glu-Trp	1117.4	64.8	1:17	yes

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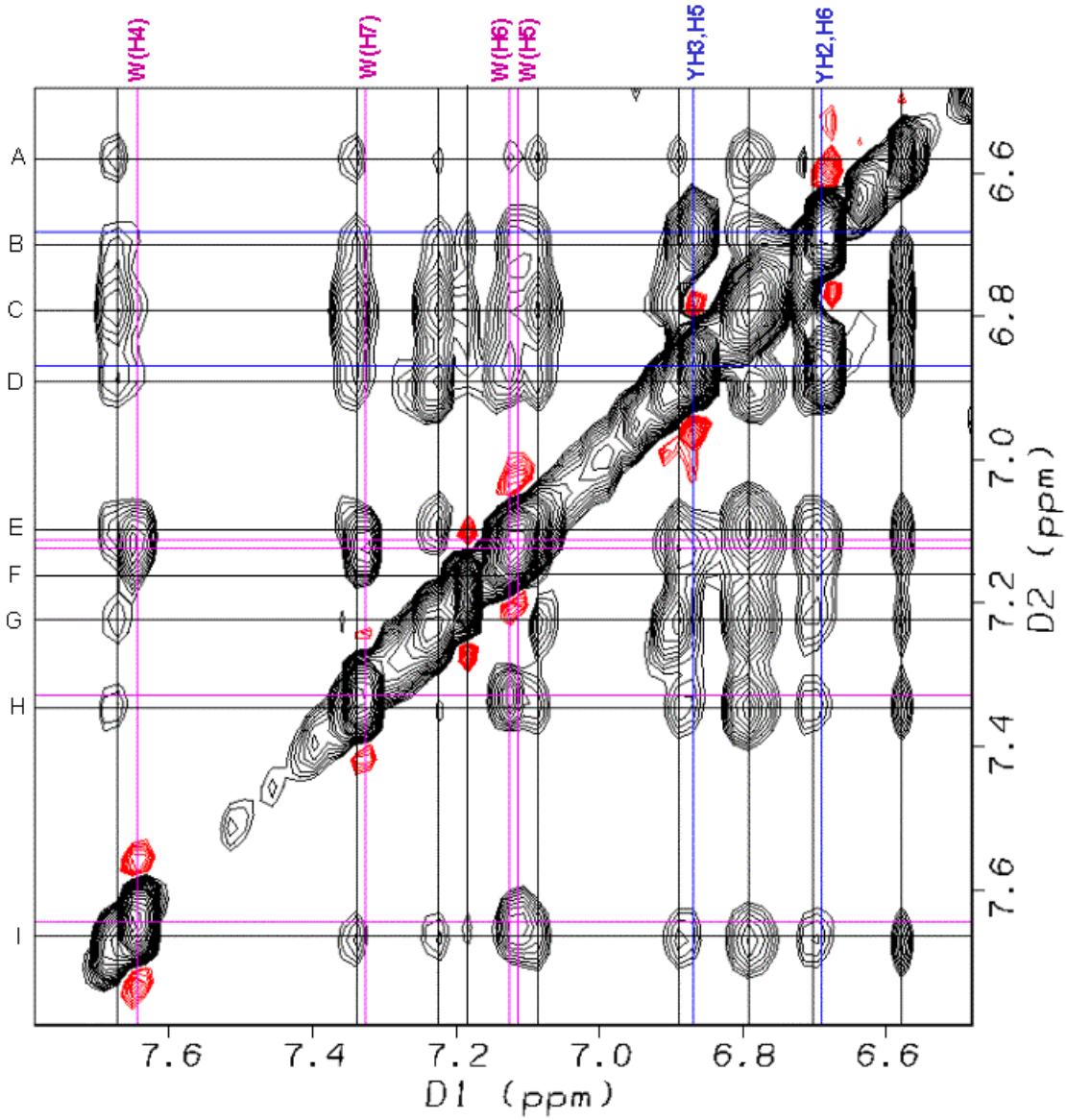

Figure S3. Expanded aromatic region of the 300 ms mixing time trNOESY spectrum at 30 ^{o}C of a mixture of lavendustin A (LAV) and Try-Glu-Trp (YEW) in the presence of TetC (black boxed region in Fig. 5). The aromatic protons of the Try and Trp residues are designated by blue and magenta lines, respectively. The ten aromatic lavendustin A protons are designated by the black lines and letters (A-I). Excluding possible NOEs that are located in overlapped regions, we can unambiguously observe the following interligand NOEs (where ever a colored and black line cross): LAV(A)-W(H6); LAV(B)-W(H5,H6); LAV(C)-W(H4, H5, H6, and H7); LAV(D)-W(H4); LAV(F)-W(H4); LAV(I)-W(H5,H6).