

Supporting Information

Characterizing Native and Hydrocarbon-stapled Enfuvirtide Conformations with Ion Mobility Mass Spectrometry and Hydrogen/Deuterium Exchange

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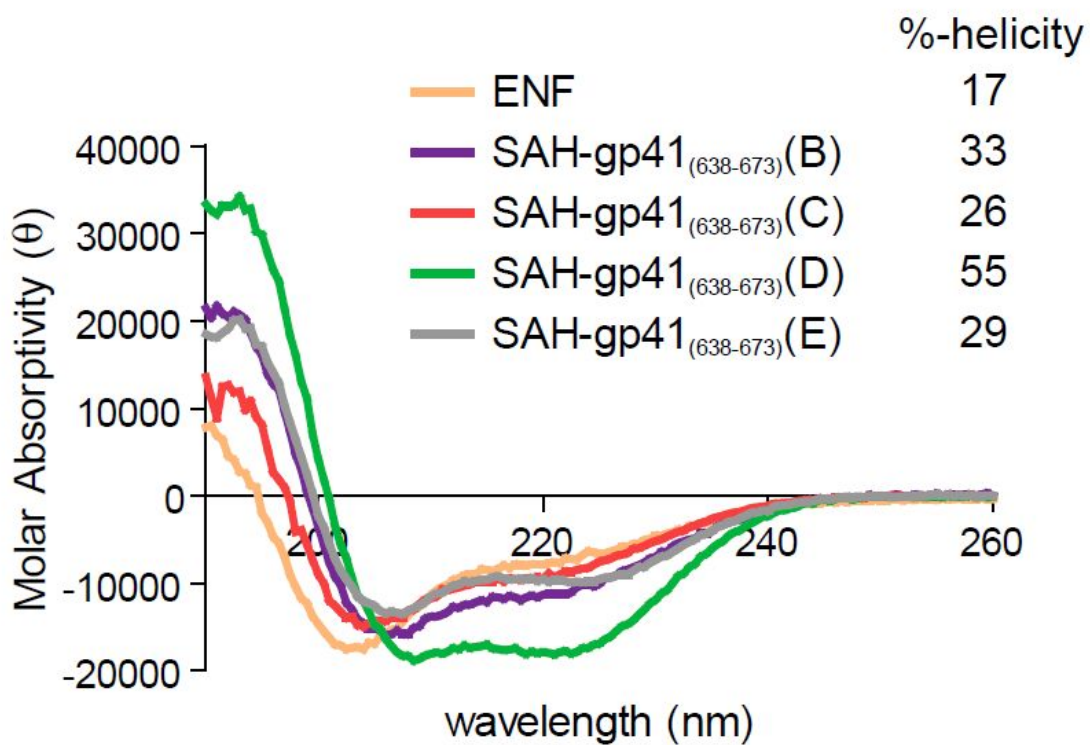


Figure S1. Circular dichroism spectra of native ENF and four single-stapled constructs. Percent helicity was calculated based on the theoretical maximum for a 36-residue peptide.

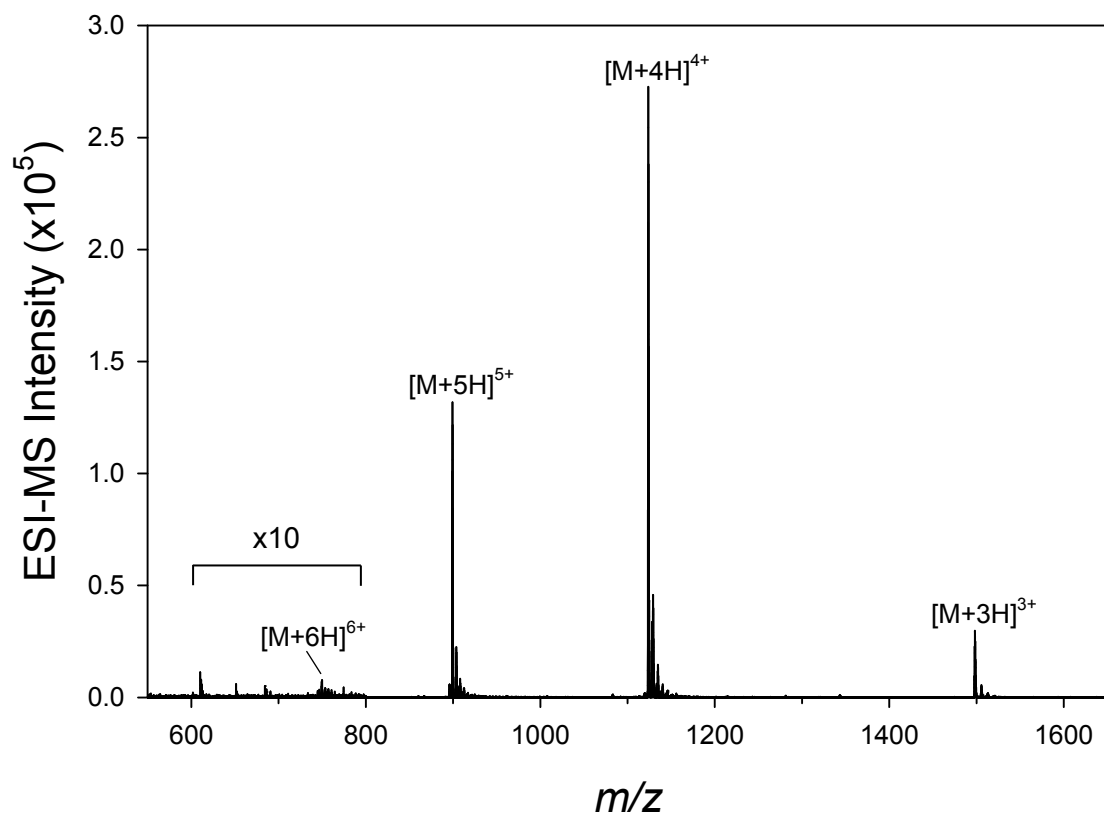


Figure S2. ESI-MS spectrum of enfuvirtide infused from 10 mM ammonium acetate. To visualize the 6+ charge state, the m/z range from 600-800 has been magnified 10-fold.

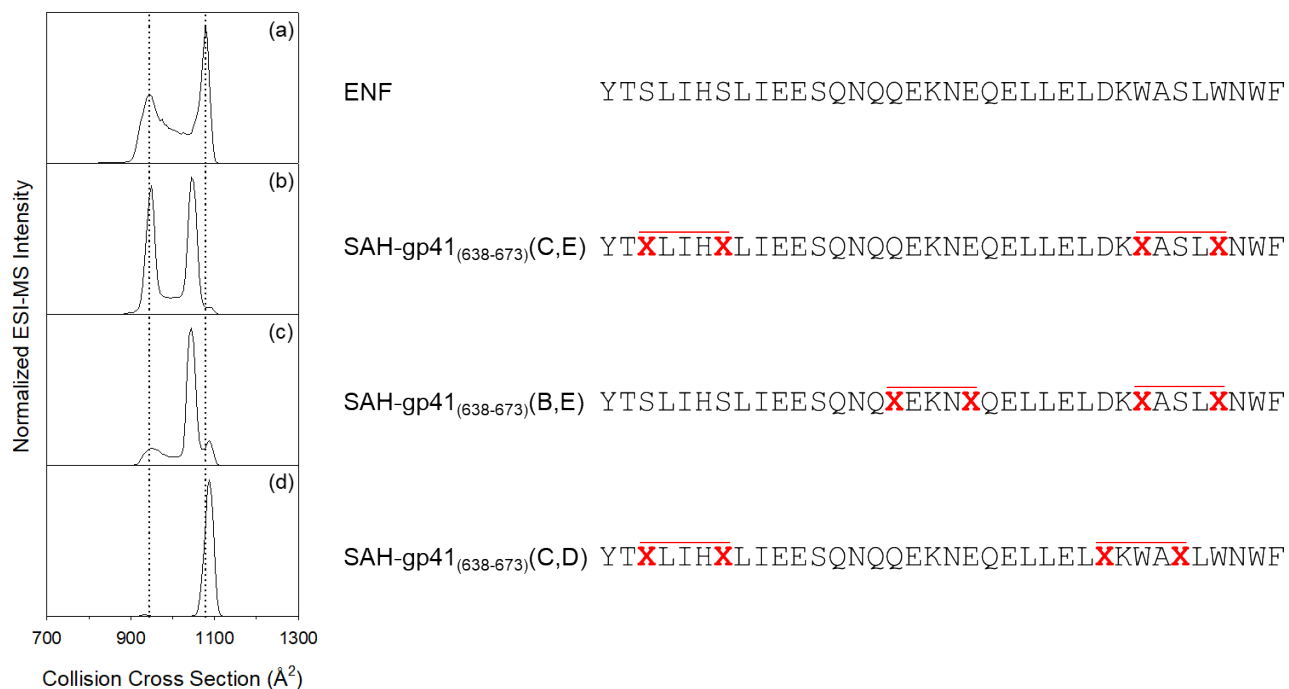


Figure S3. Collision cross section distributions ($^{TW}CCSD_{N_2}$) for (a) native ENF and (b-d) double-stapled constructs. Vertical dotted lines are meant to facilitate comparison with peak maxima observed in (a). Red lines above peptide sequences indicate $i, i+4$ hydrocarbon staples between non-native residues (X).

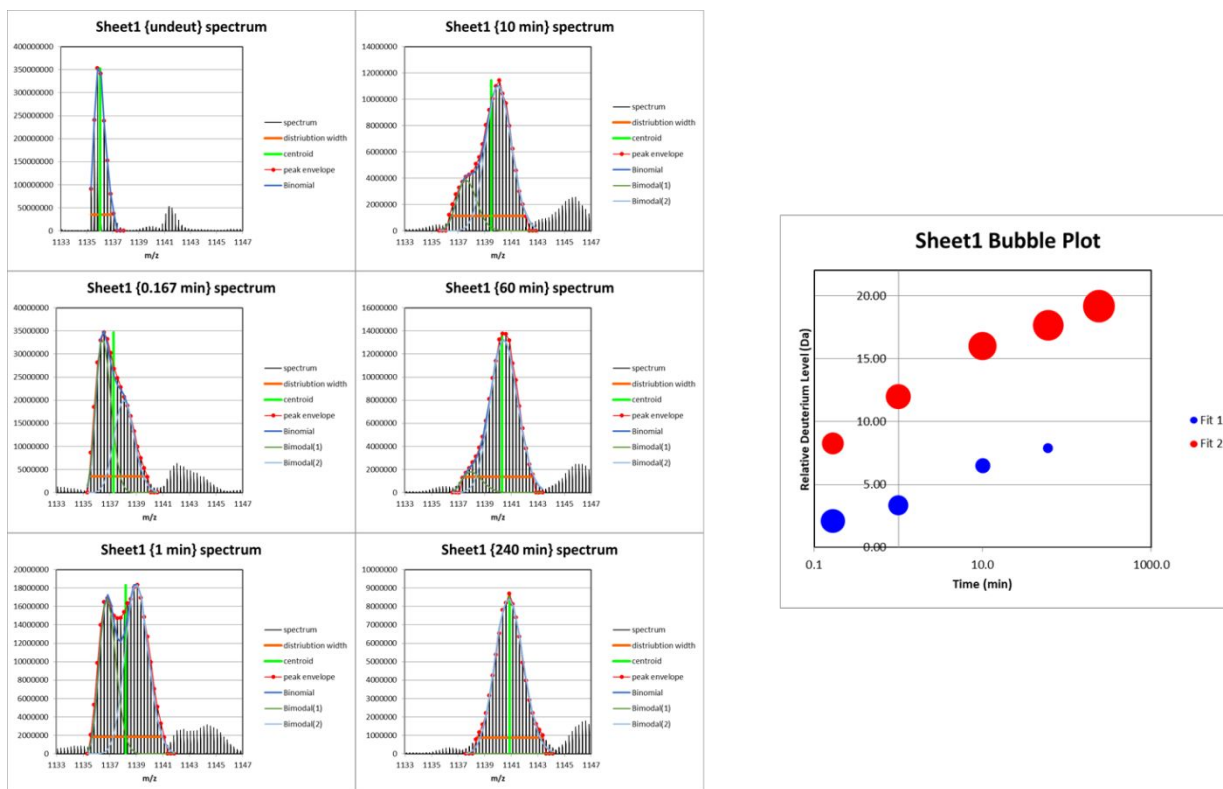


Figure S4. Isotopic envelope fitting from HXExpress for the temporal deuterium uptake of the SAH-gp41₍₆₃₈₋₆₇₃₎(D) construct. Also shown is the bubble plot, indicating the relative abundances of the low- and high-*m/z* populations as a function of exchange time. Under the labeling conditions used here, the theoretical maximum deuterium uptake is 30.24 Da.

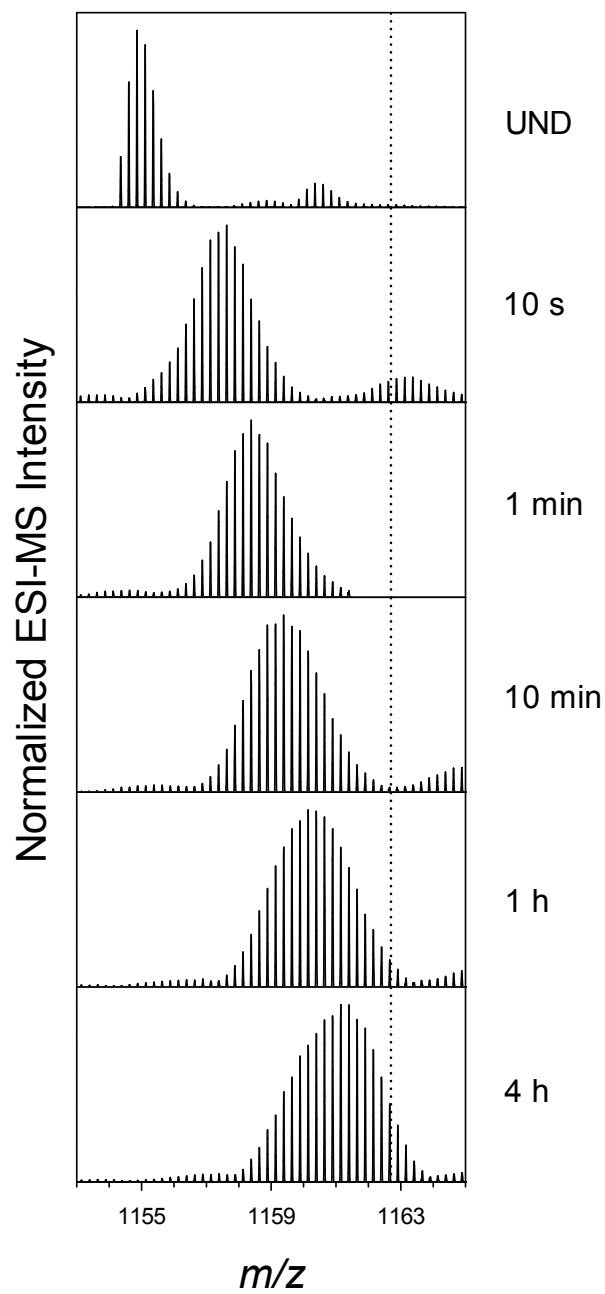


Figure S5. m/z spectra of SAH-gp41₍₆₃₈₋₆₇₃₎(C,D) double-stapled Enf construct with increasing deuterium exchange time. Vertical dotted line represents the centroid of the theoretical maximally-labelled peptide.

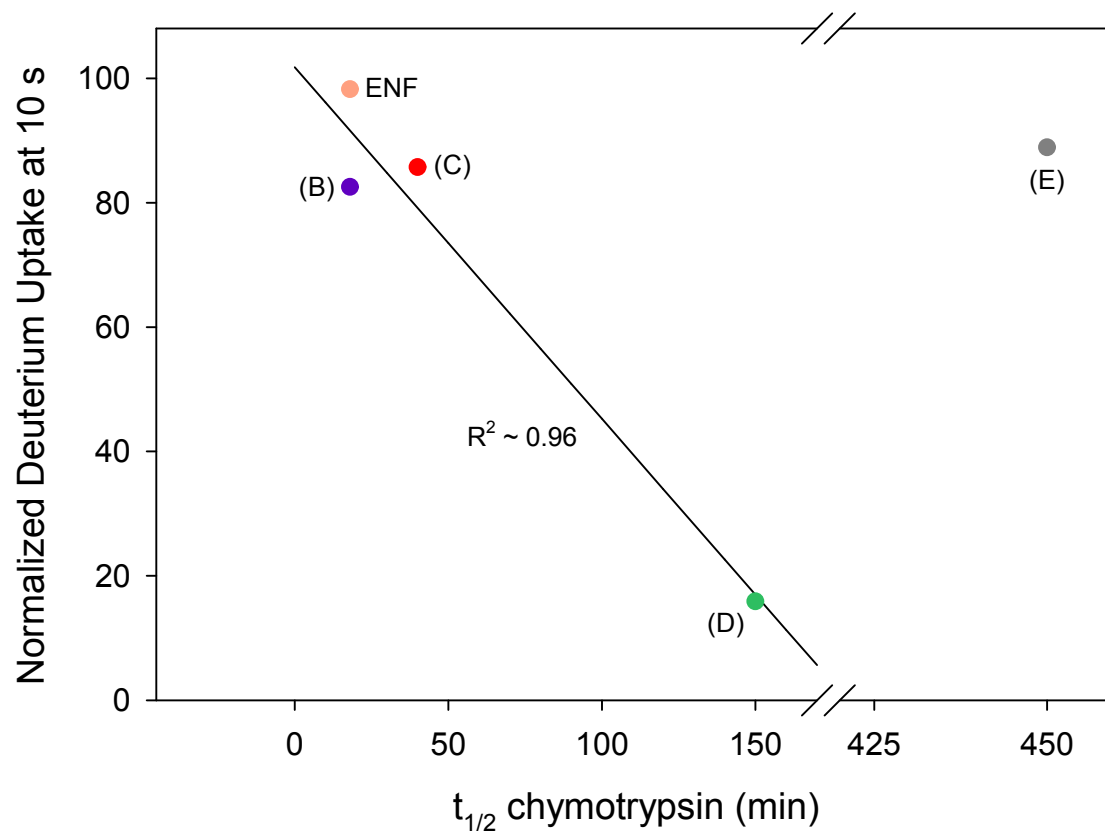


Figure S6. Relationship between deuterium uptake at 10 s and resistance to chymotrypsin digestion for native Enf and single-stapled SAH-gp41₍₆₃₈₋₆₇₃₎ constructs. Lettering refers to staple positioning as defined in Table 1 in the main text. Linear regression omitted construct (E).