SUPPLEMENTAL MATERIAL

APPLICATION OF DELTA MASS FOR THE IDENTIFICATION OF PEPTIDE:OLIGONUCLEOTIDE CROSS-LINKS BY MASS SPECTROMETRY

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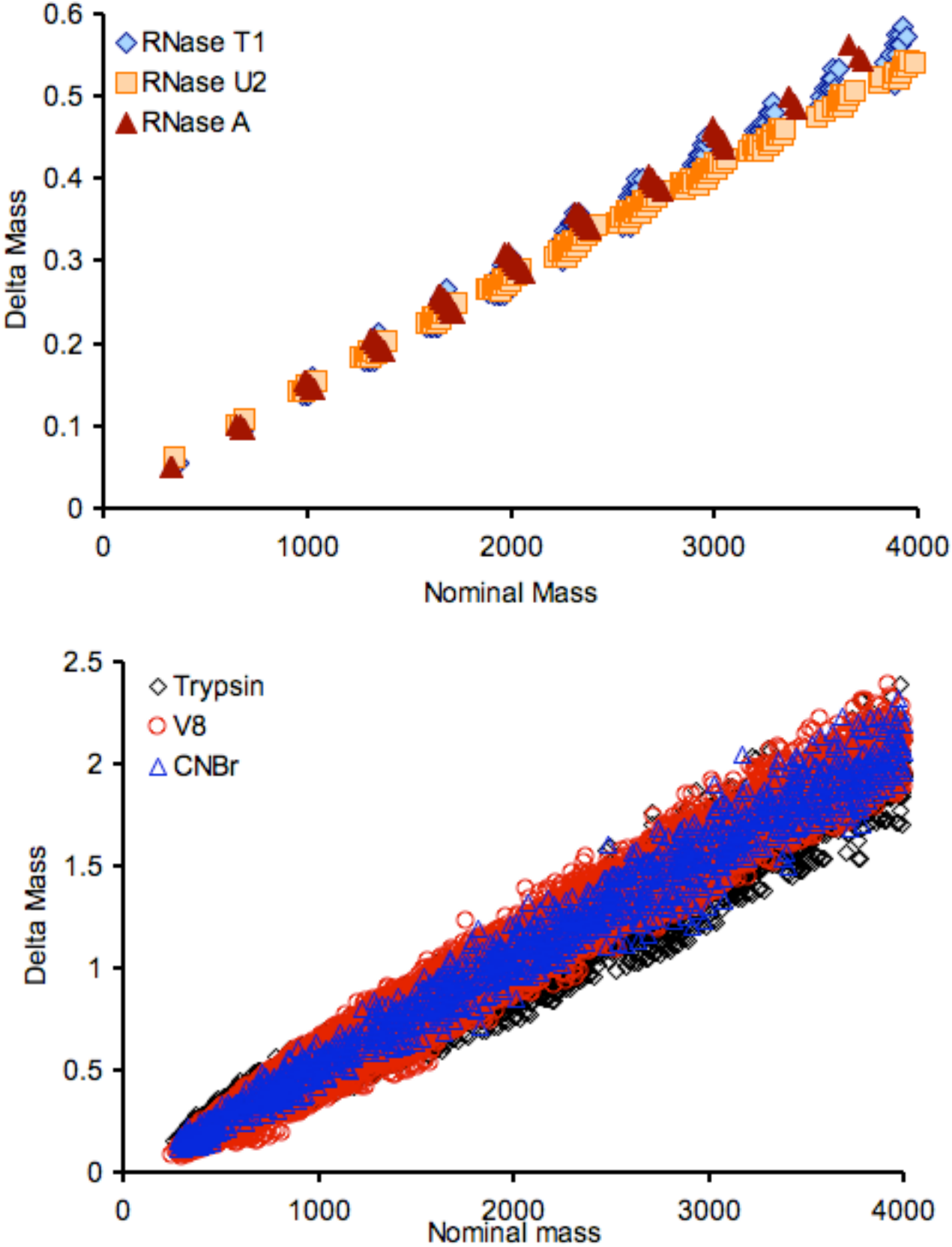

Figure S1 (a) Delta mass plot for the theoretical RNase digests of rRNA from *M. genitalium*. RNase T1, U2 and A results are shown. The type of the ribonuclease used does not affect the overall delta mass trend line for oligonucleotides. (b) Similarly, the delta mass plot for the theoretical digestion of the *M. genitalium* proteome is presented using trypsin, endonuclease V8 and cyanogen bromide for digestion. As for (a) the method for proteolytic digestion does not affect the overall delta mass trend

line for peptides. Together, these results suggest that the approach used to digest protein-nucleic acid cross-linked complexes will not limit the effectiveness of delta mass plots for identifying oligonucleotide:peptide heteroconjugates.

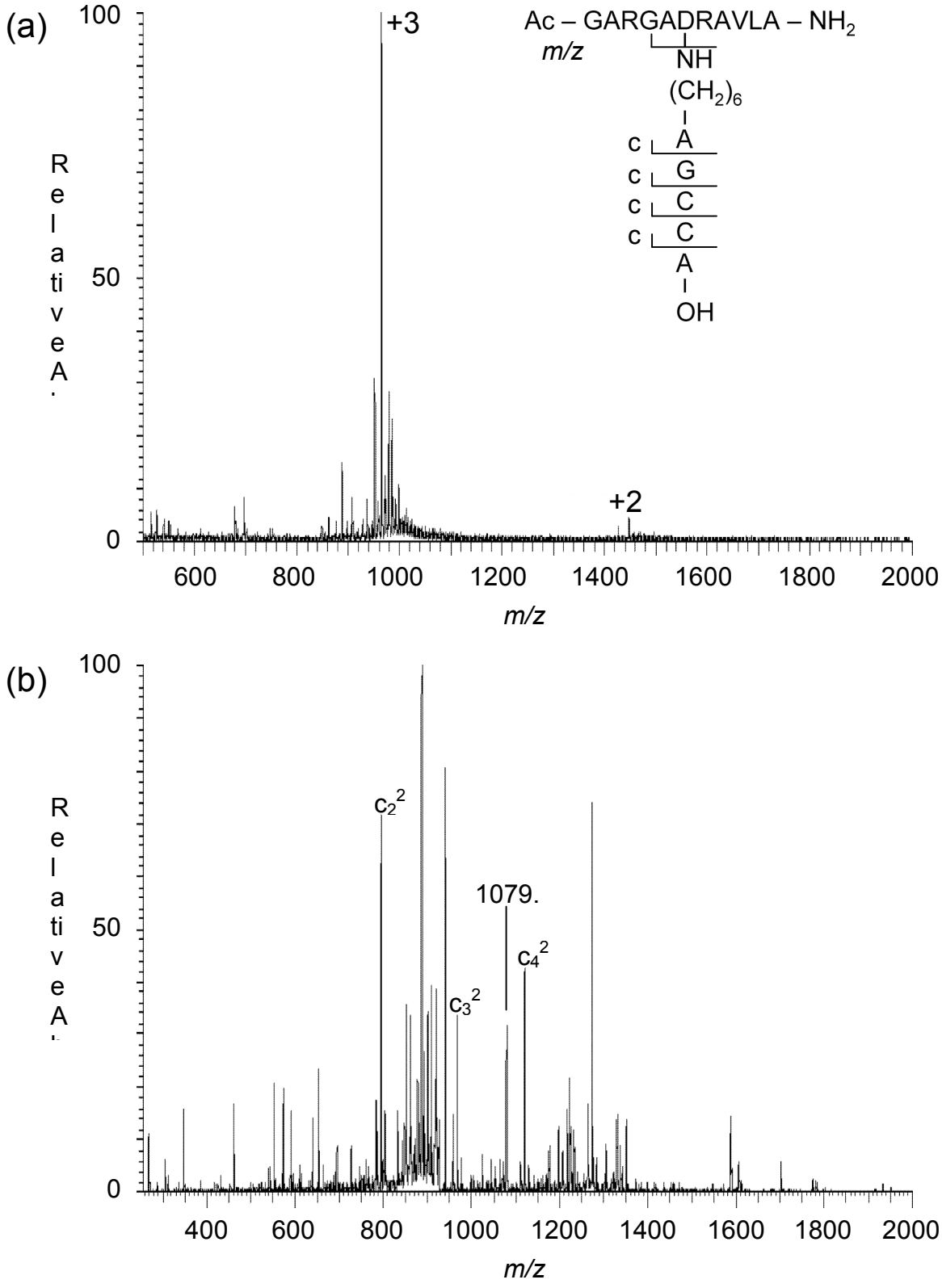

Figure S2 (a) Direct infusion electrospray ionization mass spectrometry of the heteroconjugate Ac-GARGAD(agcca)RAVLA-NH_2 in 5 mM ammonium acetate, 50 %ACN. (b) Collision-induced dissociation of the +3 charge state of the heteroconjugate. As expected, fragmentation along the oligonucleotide backbone is most prevalent (see text).

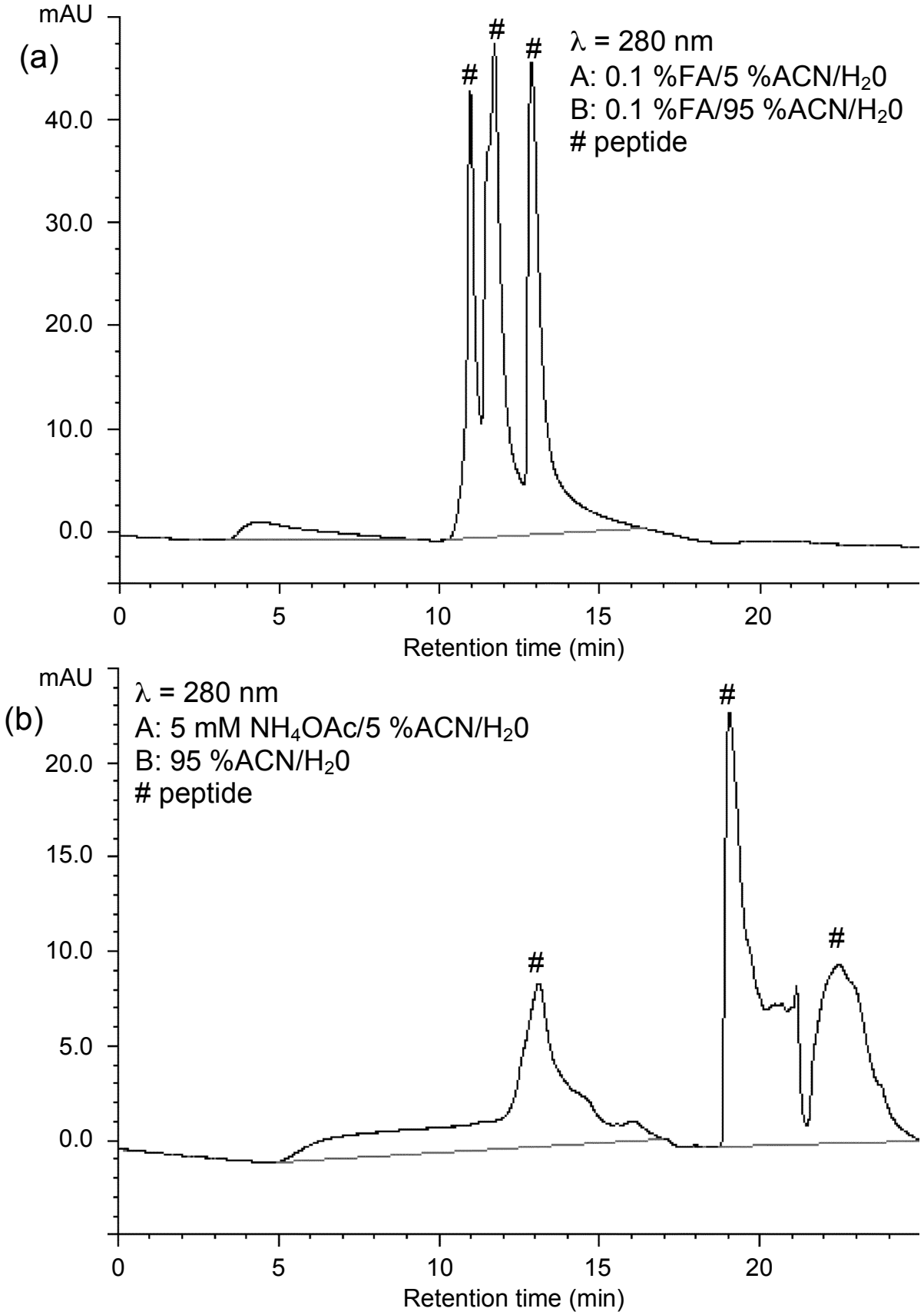

Figure S3 RP-HPLC separation on a capillary C18 column of three peptides. In (a) a gradient composed of buffer A: 0.1% FA, 5% ACN/H_2O and buffer B: 0.1% FA, 95% ACN/H_2O was used. In (b) a gradient composed of buffer A: 5 mM ammonium acetate, 5% ACN/H_2O and buffer B: 95% ACN/H_2O was used. For all chromatography, gradient conditions were 30

minute linear gradient from 20 to 95% buffer B. Peaks marked with # are the eluting peptides. Although the ammonium acetate gradient conditions are not ideal for the separation of peptides, such conditions do allow peptides to elute and, as noted in Figure 4 of the manuscript, are better suited for elution of the heteroconjugate.