Electronic Supplementary Material (ESI) for Analyst

Self reporting RNA probes as an alternative to cleavable small molecule mass tags

Jo-Anne Riley,[a] Tom Brown,[a] Nittaya Gale,[b] Julie Herniman[a] and G. John Langley*[a]

[a] *Chemistry, FNES, University of Southampton, Southampton, SO17 1BJ, UK. Tel: (0) 23 8059 2182; E-mail: gjl@soton.ac.uk*
[b] *ATDBio LTD, University of Southampton, Southampton, SO17 1BJ, UK. Fax: (0)23 8059 2991; Tel: (0)23 8059 6778; E-mail: n.gale@soton.ac.uk*

Electronic Supplementary Information

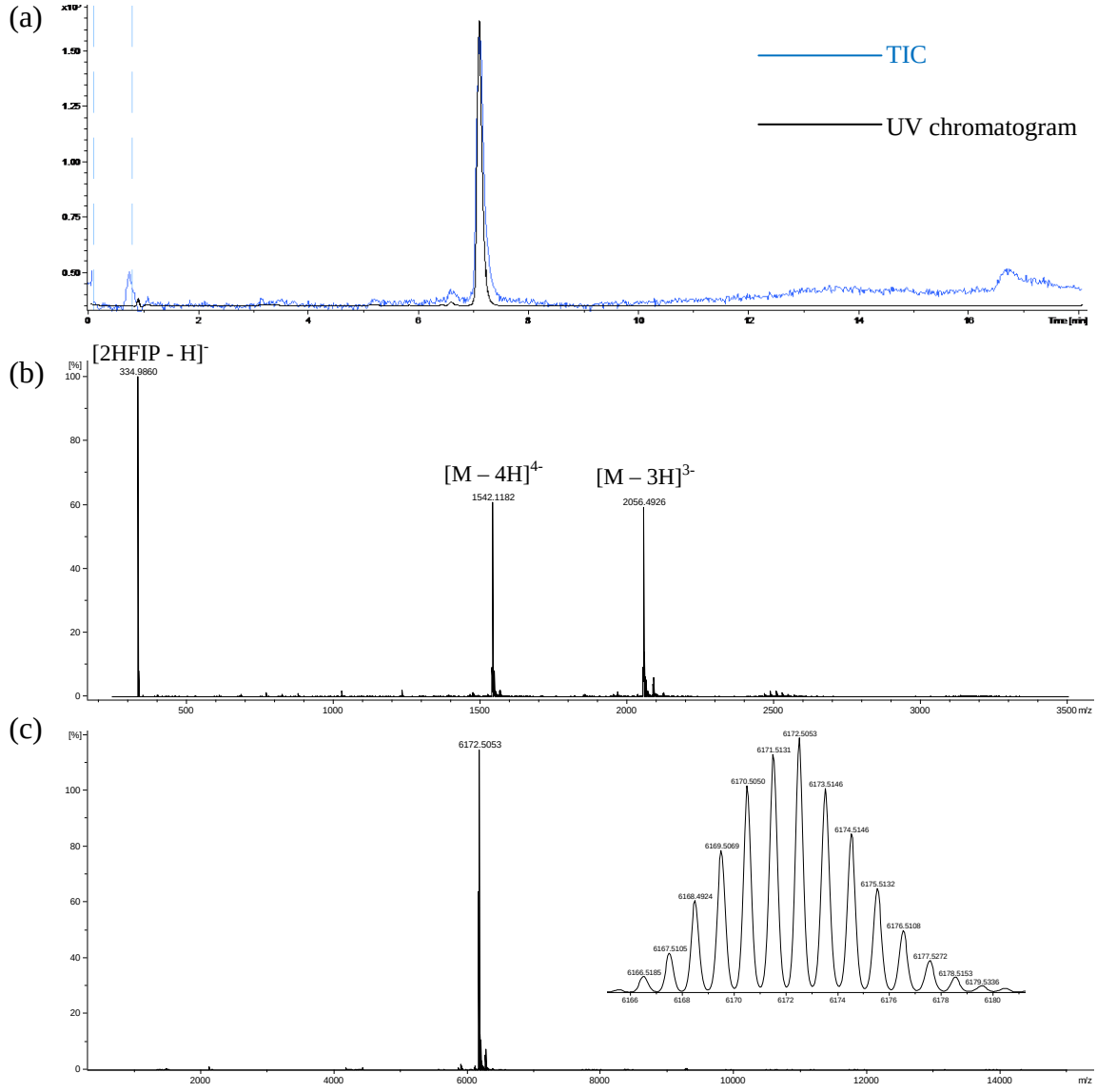

Figure S1 – HPLC-ESI MS analysis of ONT 1 prior to addition of RNase A, showing (a) TIC and UV chromatogram, (b) negative ion ESI mass spectrum and (c) MaxEnt deconvoluted spectrum

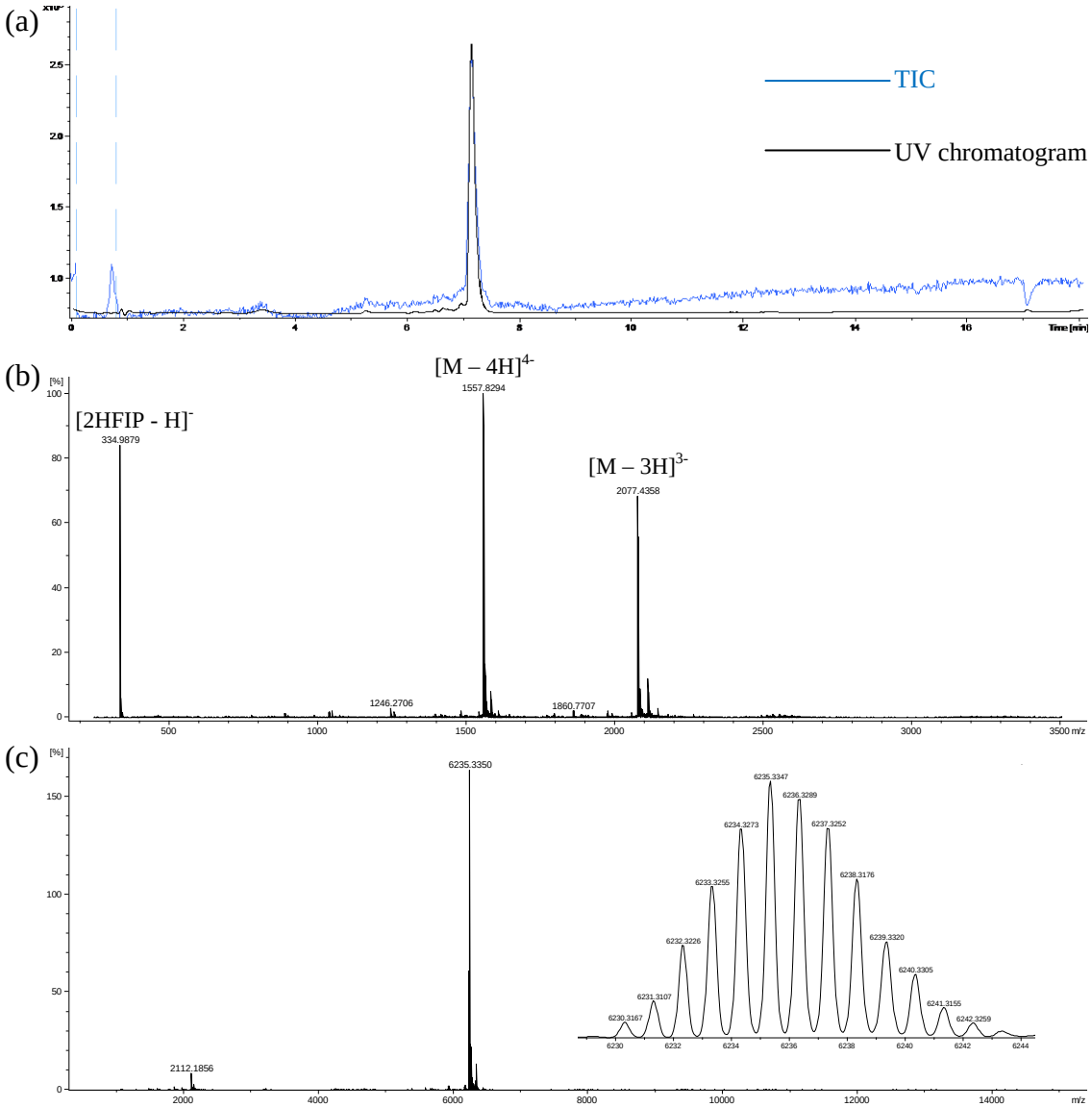

Figure S2 – HPLC-ESI MS analysis of ONT 2 prior to addition of RNase A, showing (a) TIC and UV chromatogram, (b) negative ion ESI mass spectrum and (c) MaxEnt deconvoluted spectrum.

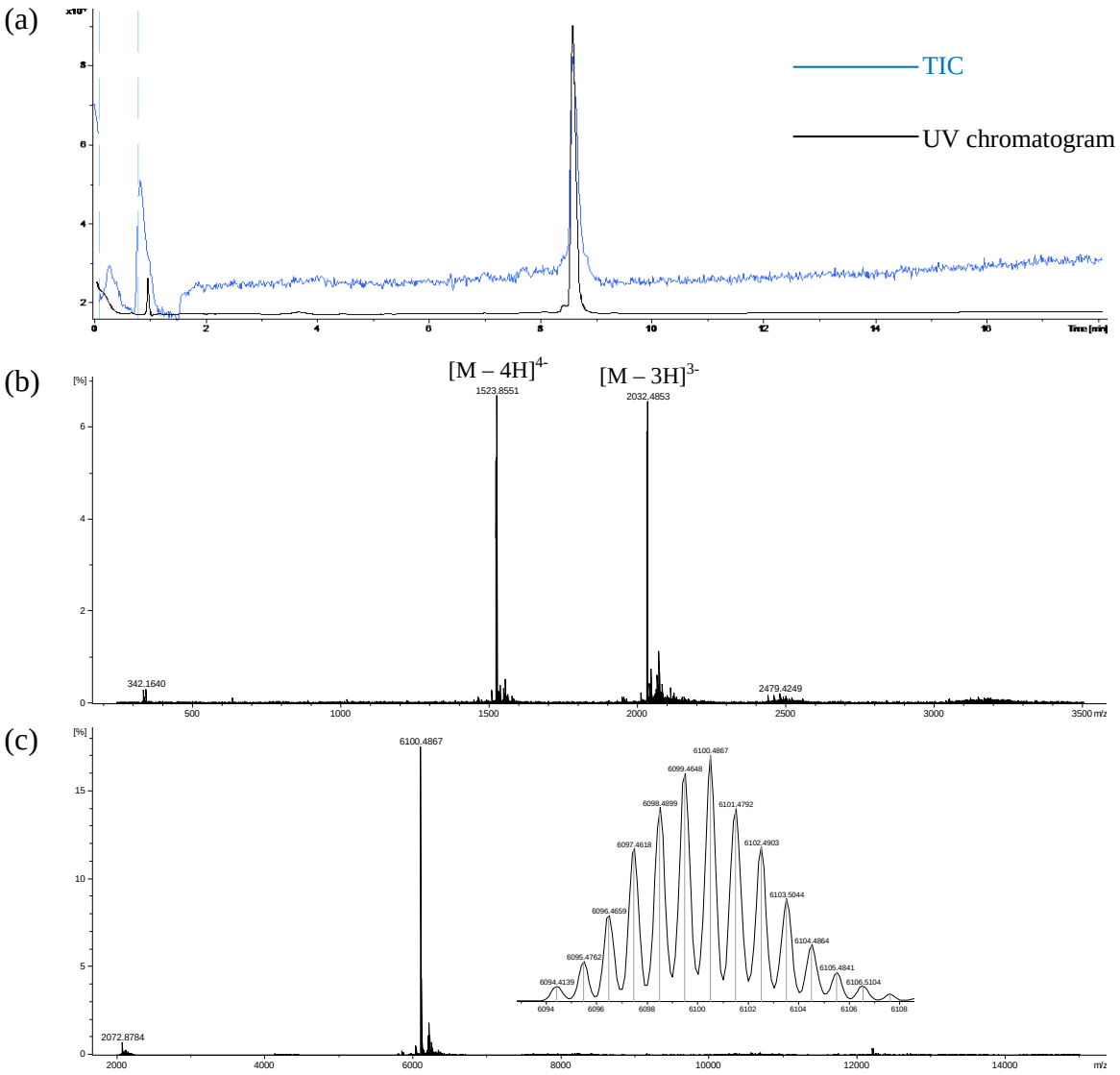

Figure S3 – HPLC-ESI MS analysis of ONT 3 prior to addition of RNase A, showing (a) TIC and UV chromatogram, (b) negative ion ESI mass spectrum and (c) MaxEnt deconvoluted spectrum.

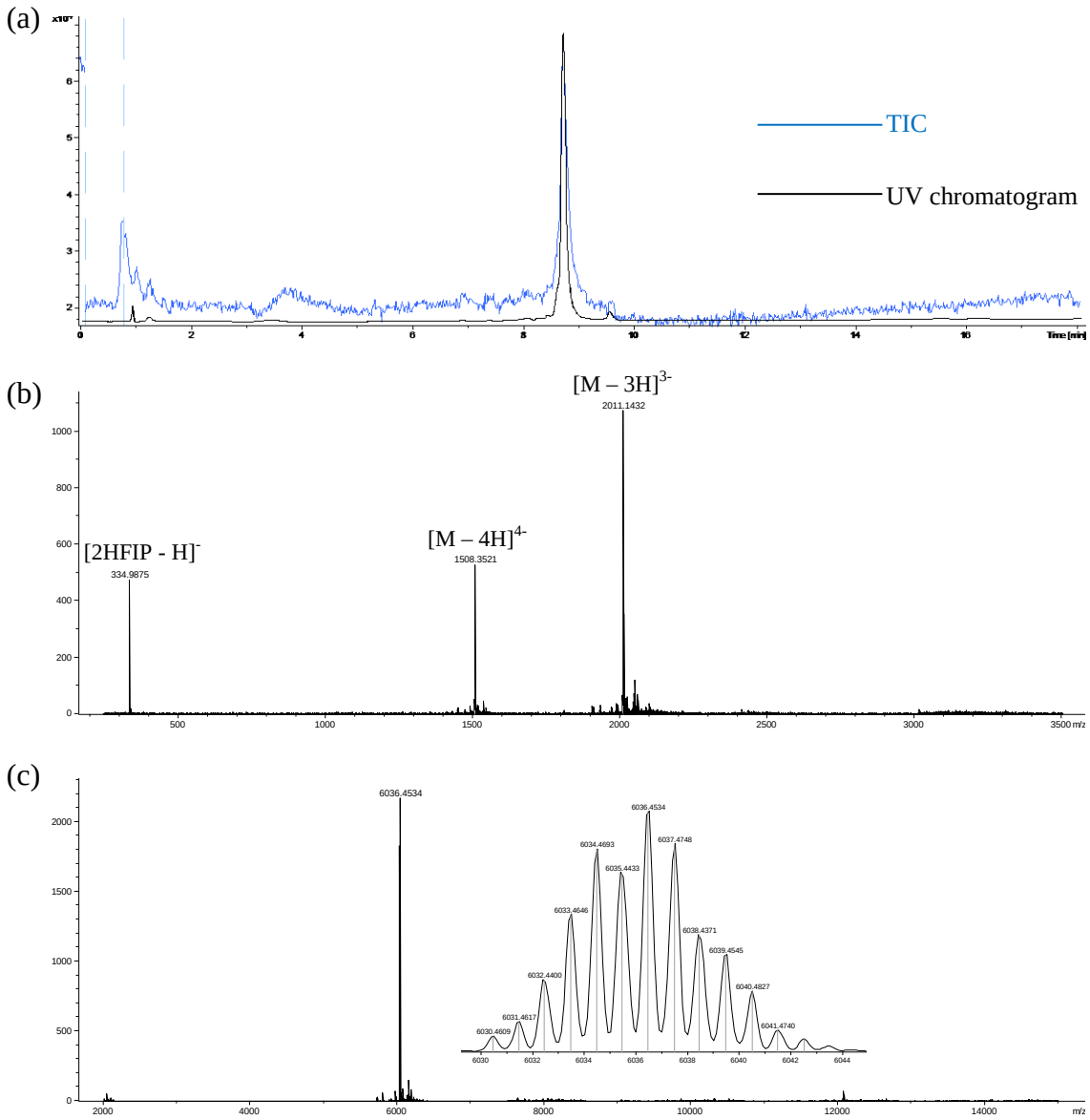

Figure S4 – HPLC-ESI MS analysis of ONT 4 prior to addition of RNase A, showing (a) TIC and UV chromatogram, (b) negative ion ESI mass spectrum and (c) MaxEnt deconvoluted spectrum.

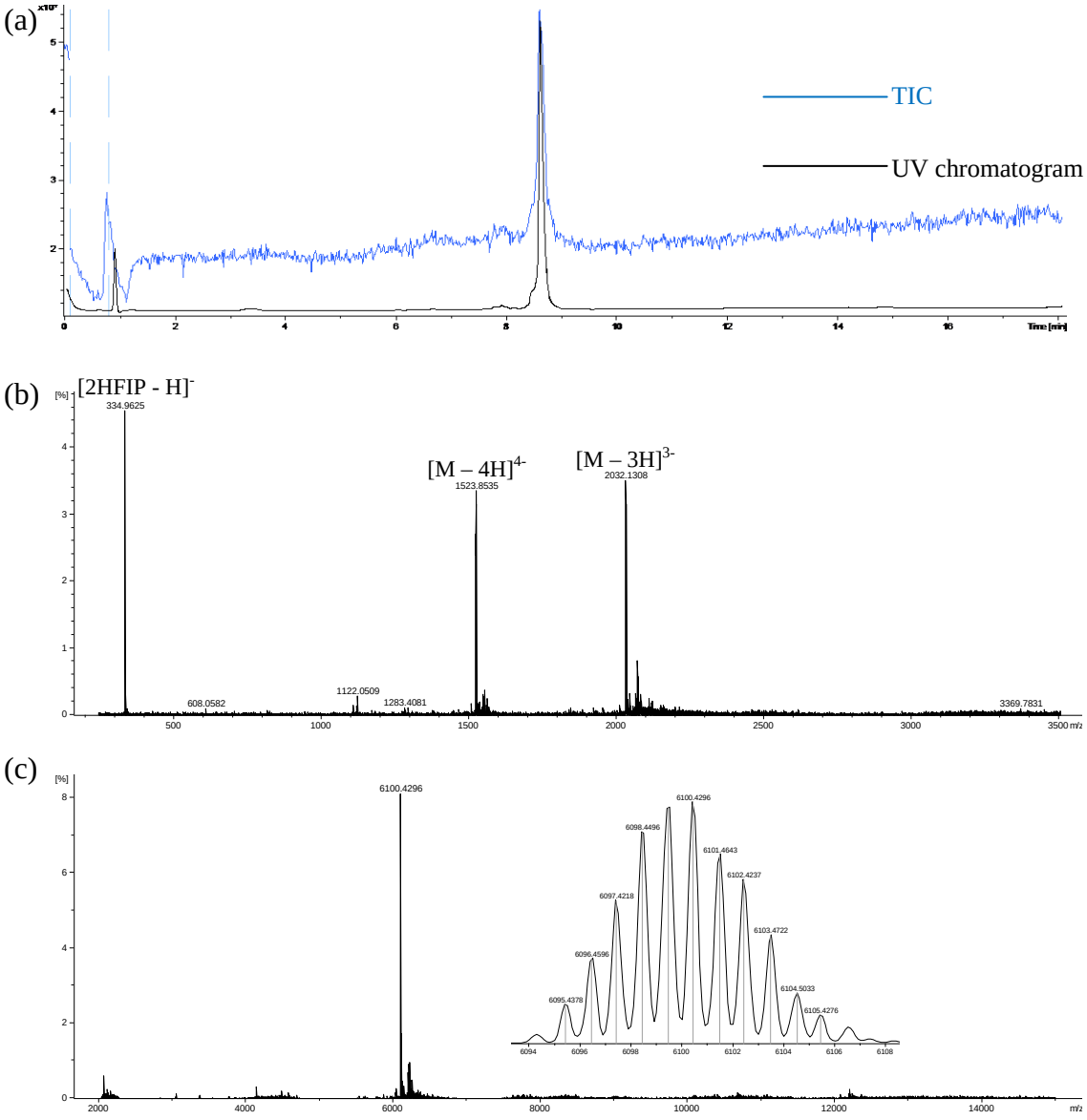

Figure S5 – HPLC-ESI MS analysis of ONT 5 prior to addition of RNase A, showing (a) TIC and UV chromatogram, (b) negative ion ESI mass spectrum and (c) MaxEnt deconvoluted spectrum.

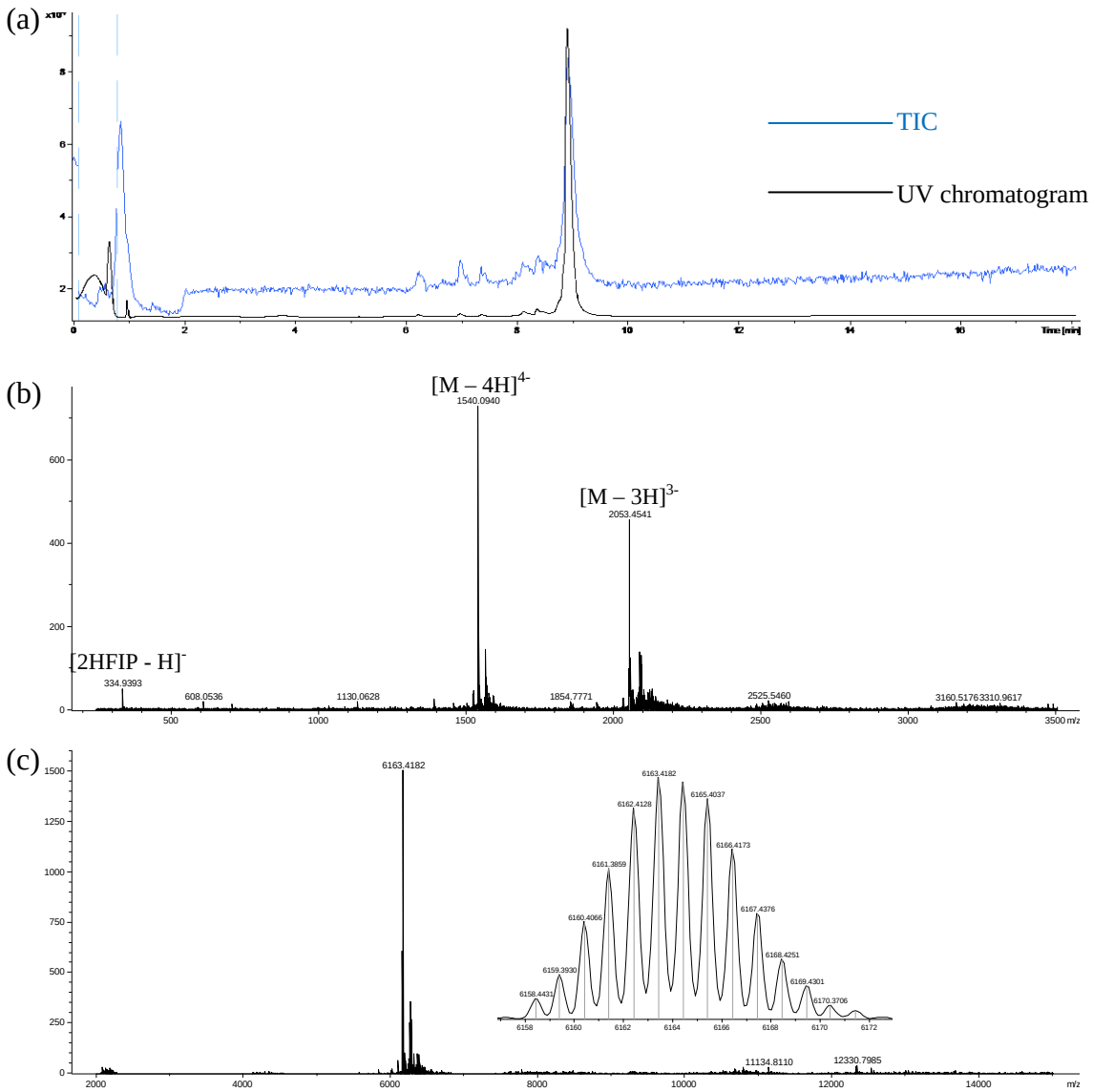

Figure S6 – HPLC-ESI MS analysis of ONT 6 prior to addition of RNase A, showing (a) TIC and UV chromatogram, (b) negative ion ESI mass spectrum and (c) MaxEnt deconvoluted spectrum.

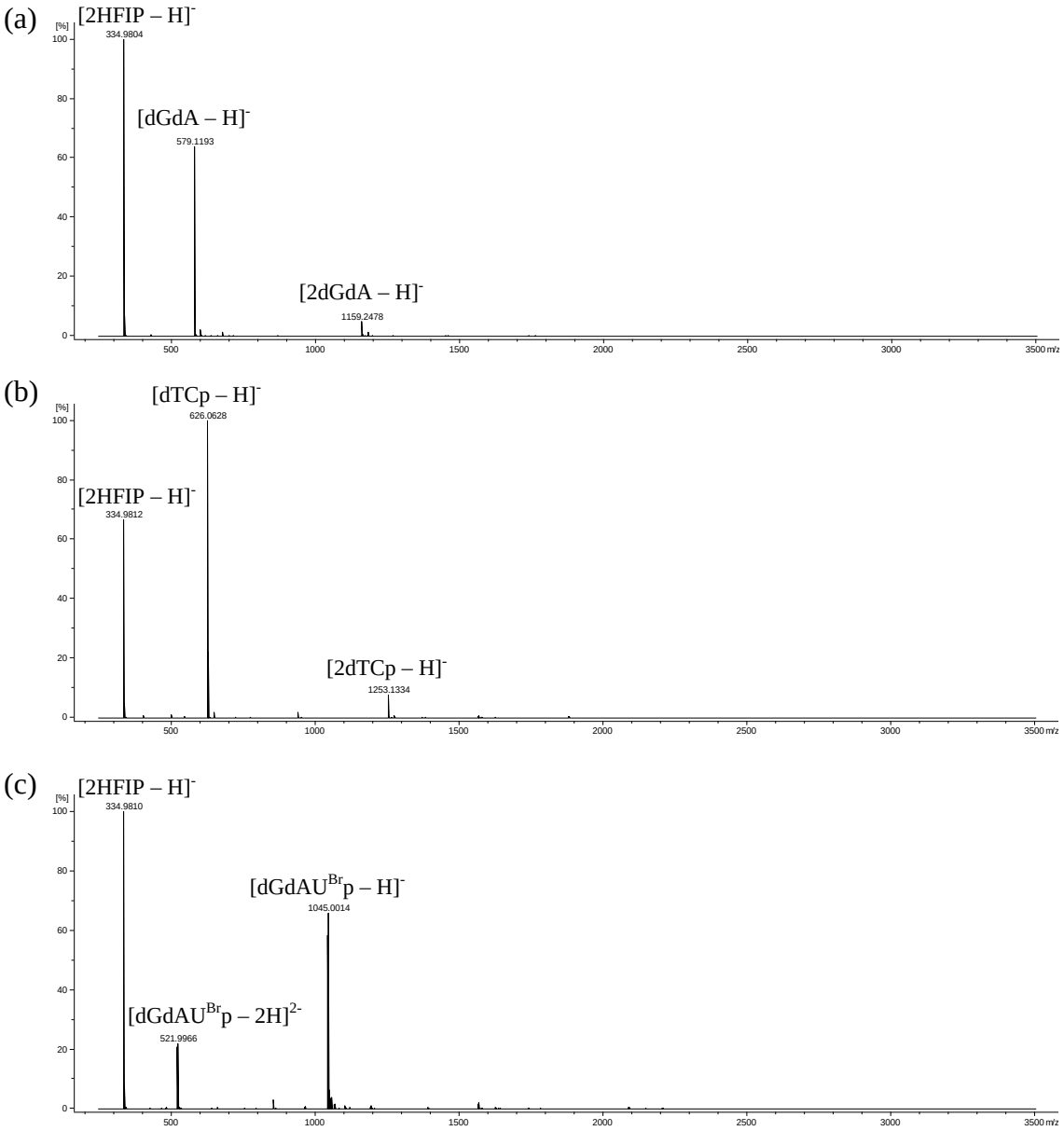

Figure S7 – Negative ion ESI mass spectra of observed digestion products of ONT 5 sampled at 5 minutes eluting at t_R (a) 1.4 min., (b) 5.4 min. and (c) 6.7 min. U^{Br} = 5-Bromouridine, HFIP = hexafluoroisopropanol.

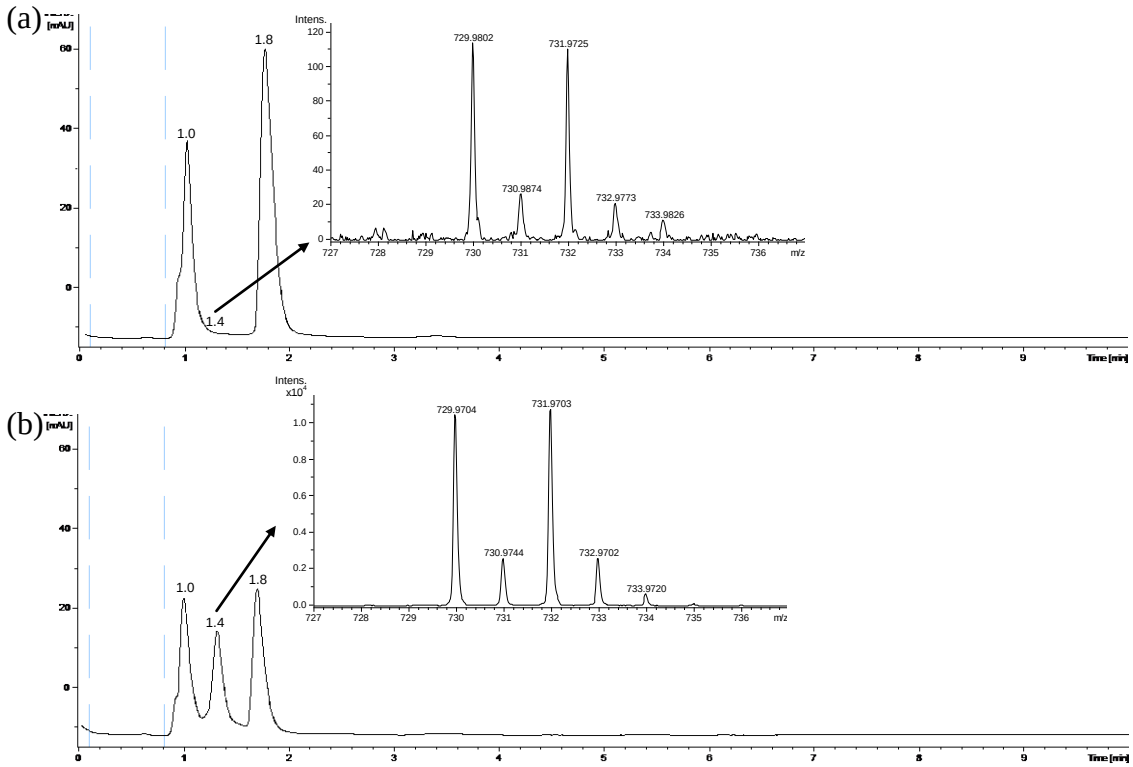

Figure S 8 – HPLC-ESI MS analysis of ONT 1 sampled at (a) 5 minutes and (b) 24 hours showing UV chromatograms and negative ion ESI mass spectra of the brominated overdigestion product AU^{Br}p. U^{Br} = 5-bromouridine and p = 3′-phosphate. N.B. mobile phase gradient normally used for analysis of intact RNA was used for this analysis.

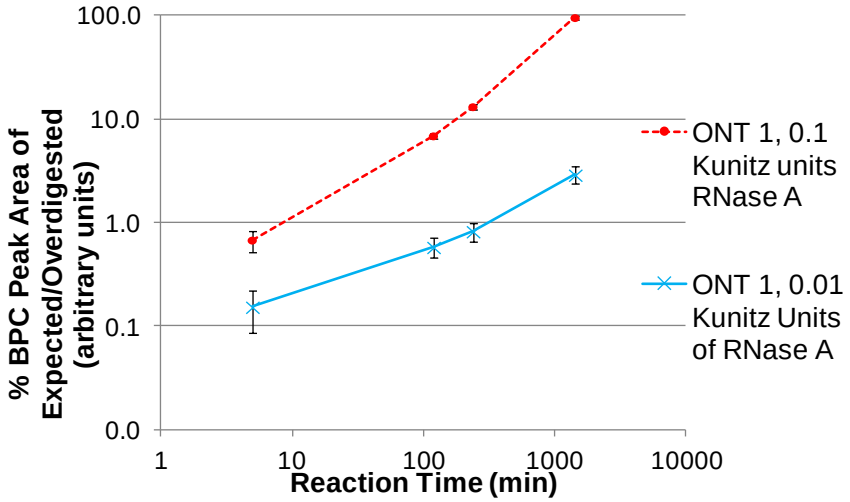

Figure S 9 – A graph showing the peak area of AU^{Br}p compared to AAU^{Br}p for ONT 1 under different assay conditions. Error bars show ± 1 standard deviation from triplicate results. d = deoxynucleotide, p = 3′-phosphate

Figure S10 – RNase A expected cleavage sites (green solid line) and unexpected, non-specific cleavage sites (red dashed line) for (a) ONT 1 and (b) the *n*-3 failure sequence of ONT 4, highlighting the proposed origins of the unexpected dinucleotide products. U^{Br} = 5-bromouridine