Appendix 2B

Supplemental Figures

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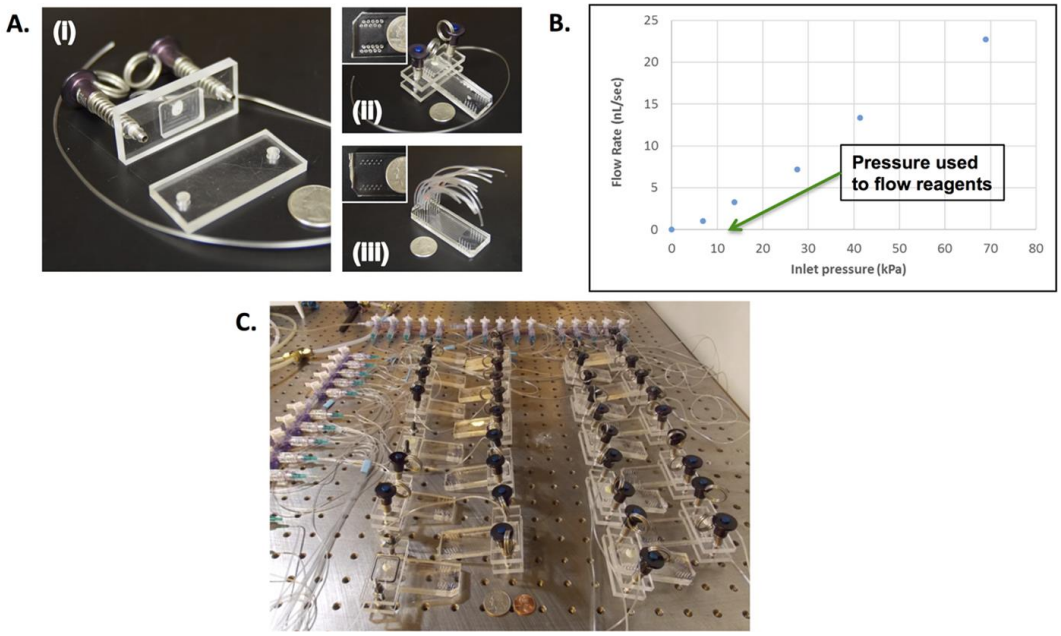


Figure A2B.1. High throughput patterning of DNA barcode chips. A. (i) Solution loading device design and flow patterning set-up with (ii) the solution loading device used in this work and (iii) the pins and tubing used for flow-patterning in previous work. B. Measured flow rates under various inlet pressures using the solution loading device. C. Simultaneous flow patterning of 21 DNA barcode chips.

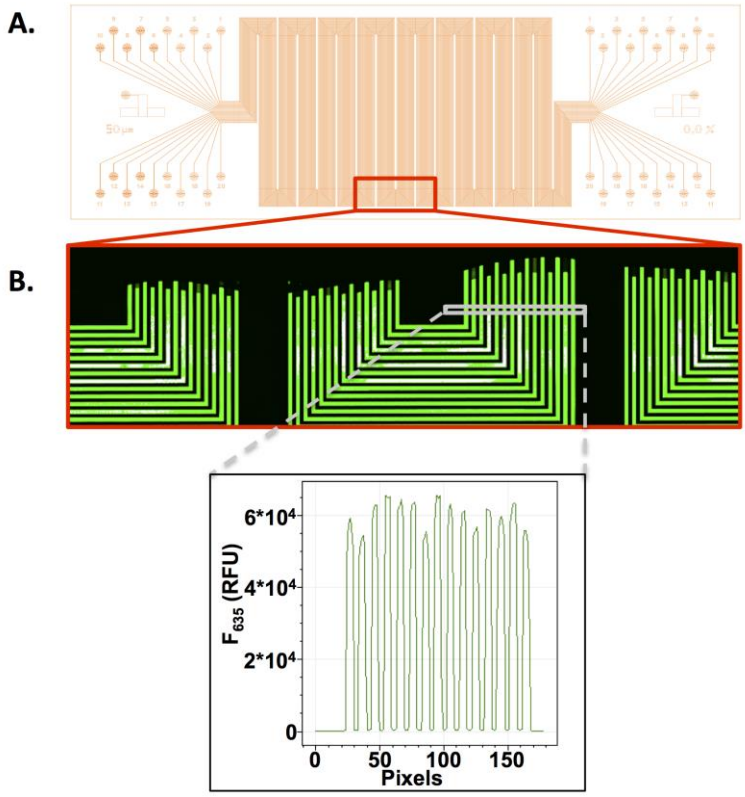


Figure A2B.2. DNA barcode chip layout and validation. A. The 50 µm barcode chip layout, which encompasses the entire length of a 3" microscope slide. Input and outputs of the serpentine microchannels are at the right and left sides. B. An image and digitized line scan of a barcoded region of the slide. The white box in the image outlines a region that is approximately 50 µm x 2 mm wide.

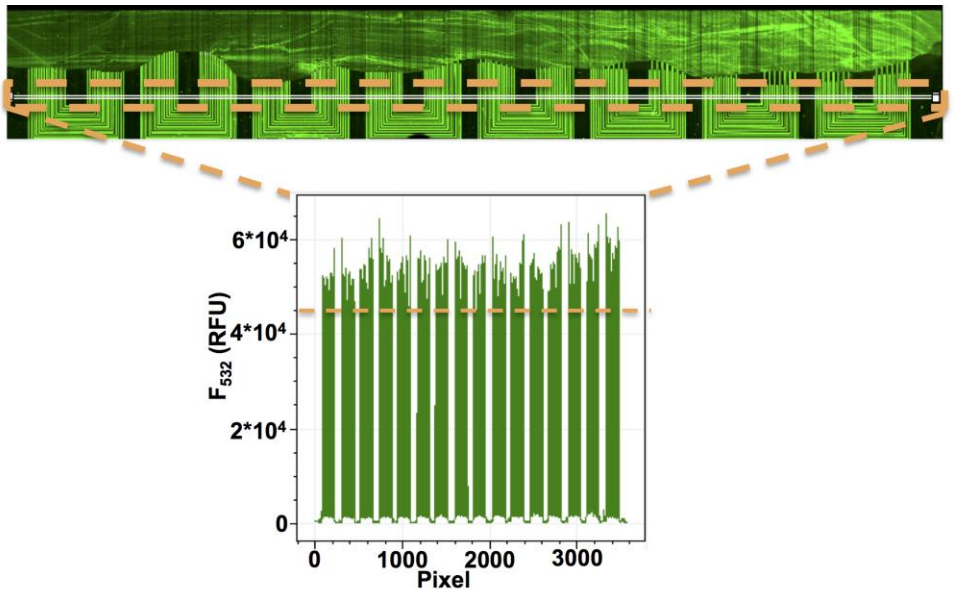


Figure A2B.3. Comparison of barcode quality across an entire microchip. Measuring the barcode quality across the entire barcode region (about 3.8 cm) reveals that all of the barcode lanes meet the minimum F_{532} requirement for good levels of ssDNA patterning as highlighted by the dashed line on the graph.

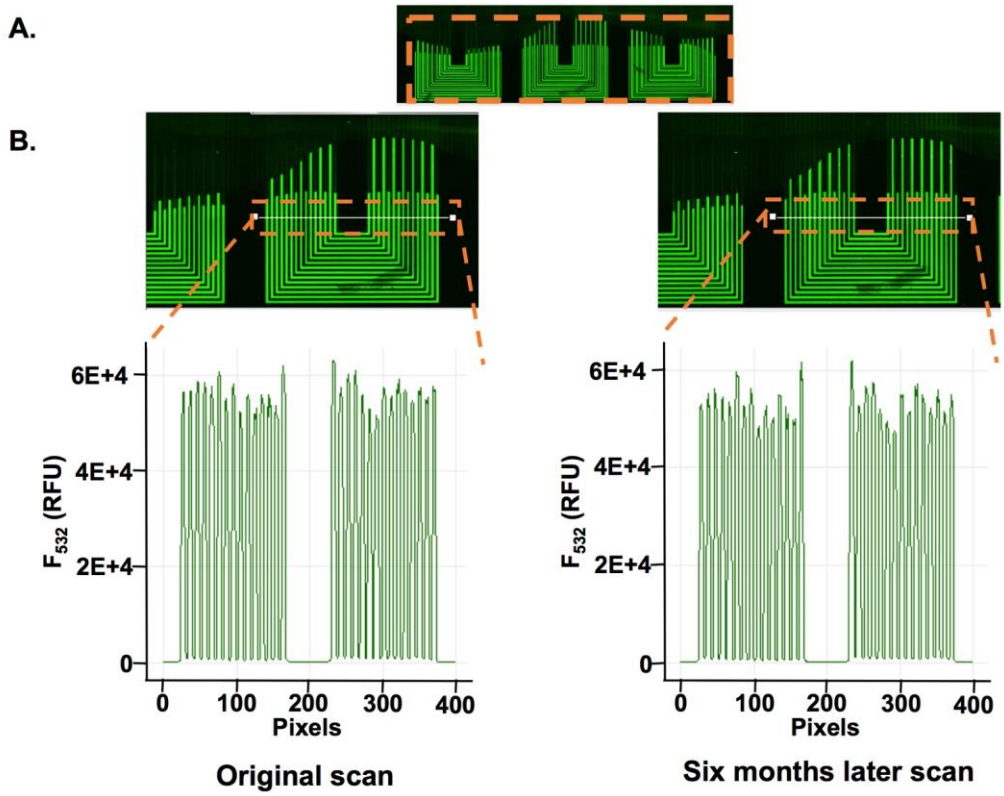


Figure A2B.4. Probing barcode stability. A. The portion of the validation region used to generate Table A2C.4. B. A comparison of F_{532} output for two DNA barcode sets in the validation region with six months between each scan.

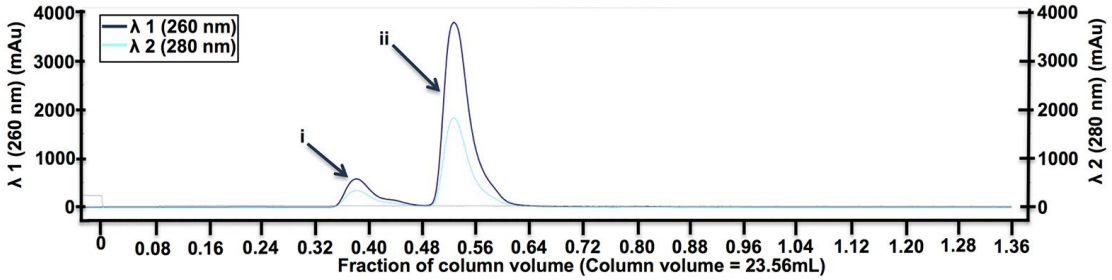


Figure A2B.5. Representative SAC-DNA FPLC trace. The FPLC trace for B' SAC-DNA. The SAC-DNA elutes first (peak labeled "i") followed by the excess unconjugated B' ssDNA (peak labeled "ii").

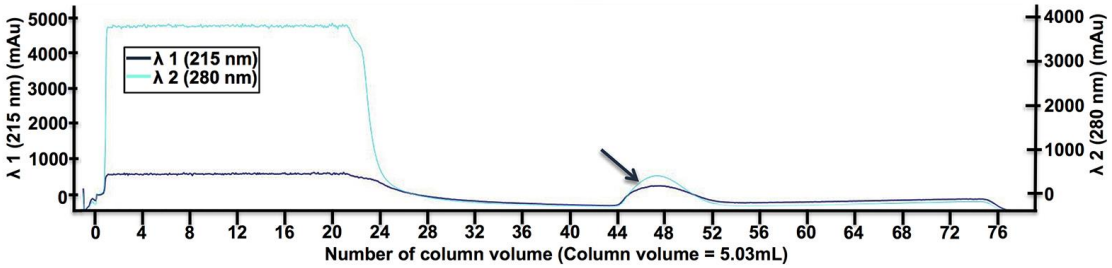


Figure A2B.6. FPLC trace of wild-type KRas protein. The FPLC trace for wild-type KRas protein with the protein peak highlighted with a black arrow.

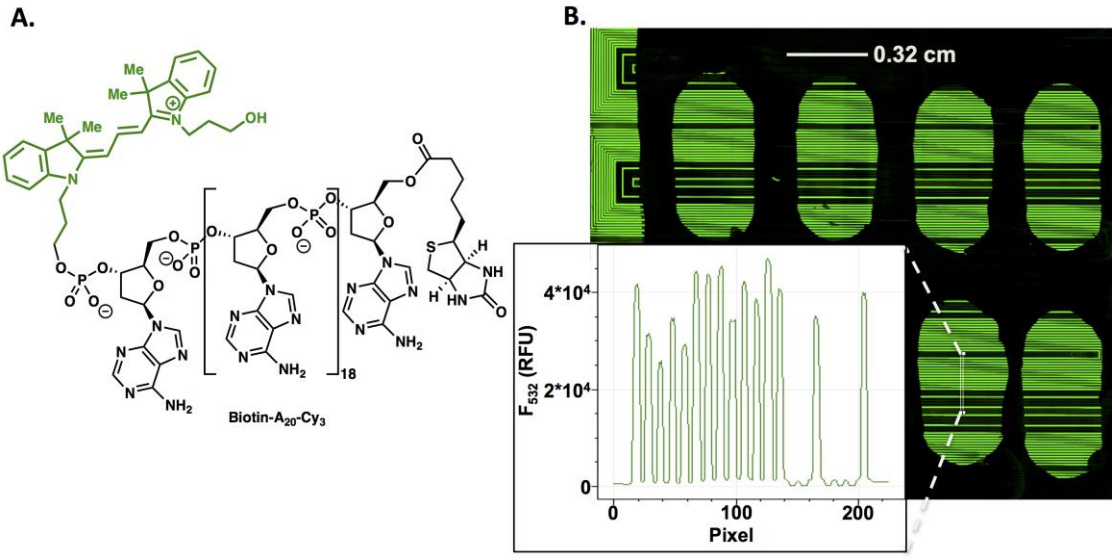


Figure A2B.7. Biotin binding evaluation of the full SAC-DNA set with a Biotin* probe on the barcoded rapid assay platform. A. The Biotin* probe used to perform the biotin binding affinity test for the DESL set using the B-RAP technology. B. The output of the biotin binding test on the DNA barcode.

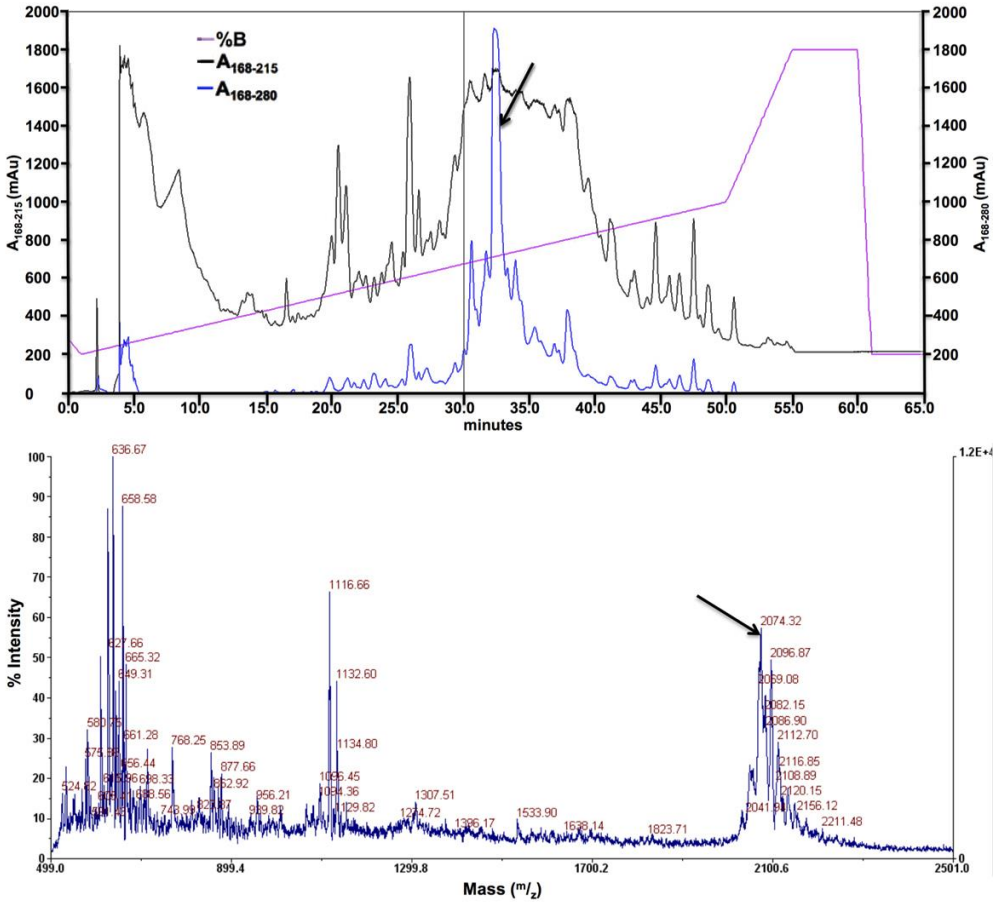


Figure A2B.8. The characterization data for SynEp1. SynEp1 FDEYD[P→$^{4\text{-}N3}$P]TIEDSY-PEG$_4$-Biotin: $C_{90}H_{128}N_{20}O_{33}S$. (Top) The semi-preparative HPLC trace for SynEp1 with the peak containing the correct mass indicated by an arrow. (Bottom) The MALDI-TOF spectra for **SynEp1** with the peak indicated with an arrow corresponding to [M+Na], expected [M+Na]=2071.188 amu.

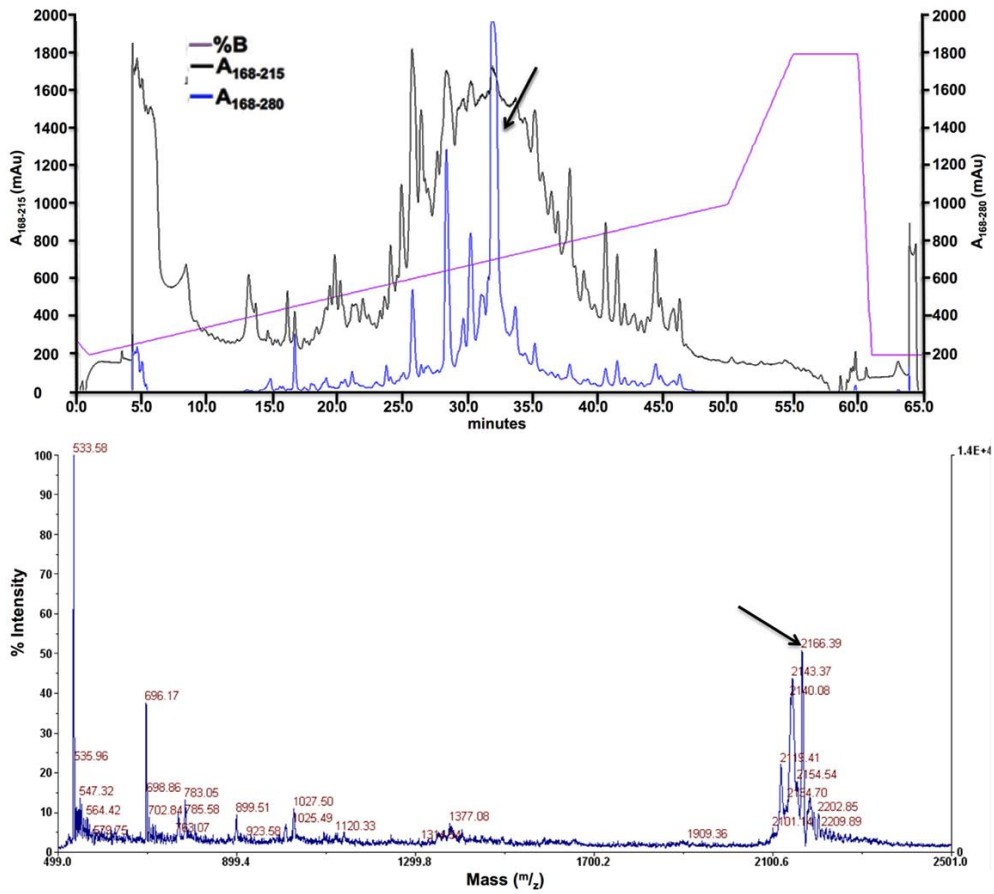


Figure A2B.9. The characterization data for scrambled SynEp1. Scrambled **SynEp1** [P→$^{4\text{-}N3}$P]EYDSIDDEFYT-PEG$_4$-Biotin: $C_{90}H_{128}N_{20}O_{33}S$. (Top) The semi-preparative HPLC trace for scrambled **SynEp1** with the peak containing the correct mass indicated with an arrow. (Bottom) The MADLI-TOF spectra for scrambled **SynEp1**. The peak indicated with an arrow corresponds to [M+2Pip-3 OH+H], expected [M+2Pip-3 OH+H]=2167.030 amu, which results from multiple piperidine and aspartamide adducts forming on the oligopeptide.

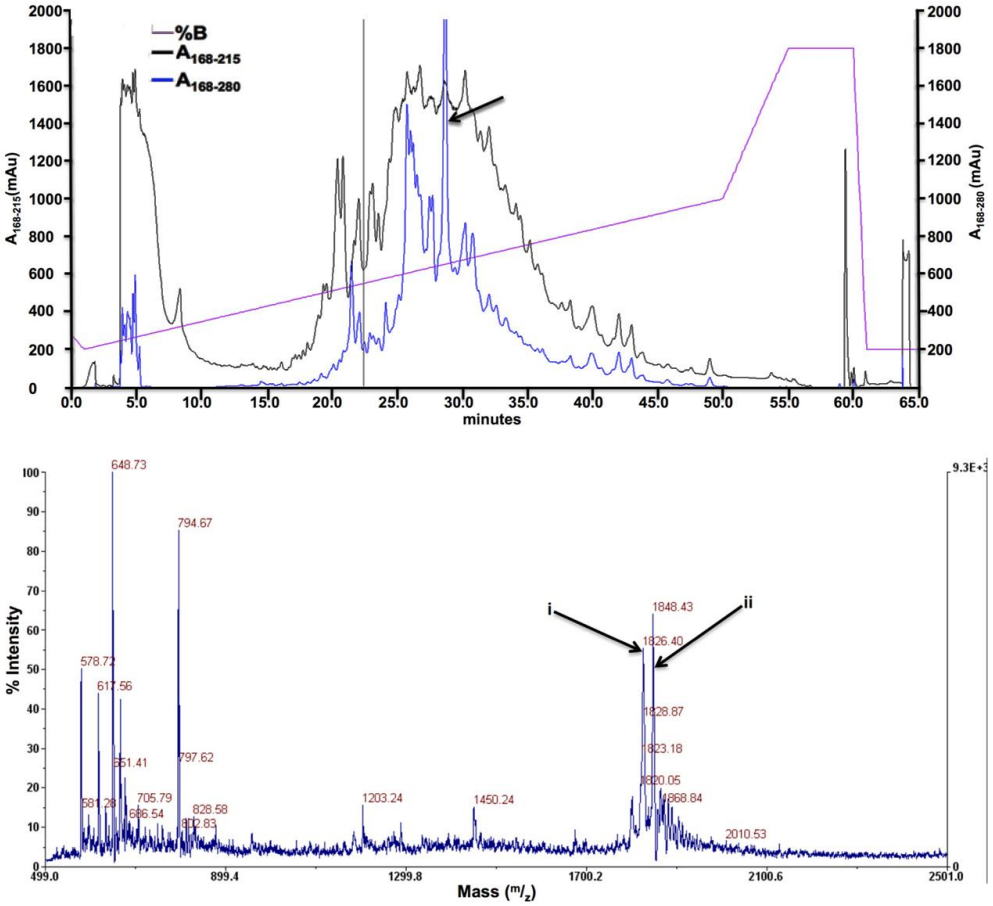


Figure A2B.10. The characterization data for SynEp2. SynEp2 [I→$^{4\text{-}N3}$K]LDTAGQEEYS-PEG$_5$-Biotin: $C_{77}H_{124}N_{20}O_{29}S$. (Top) The semi-preparative HPLC trace for **SynEp2** with the peak containing the correct mass indicated by an arrow. (Bottom) The MALDI-TOF spectra for **SynEp2**. The peak labeled "i" corresponds to [M+H], expected [M+H]=1825.864 amu, and the peak labeled "ii" corresponds to [M+Na], expected [M+Na]=1847.846 amu.

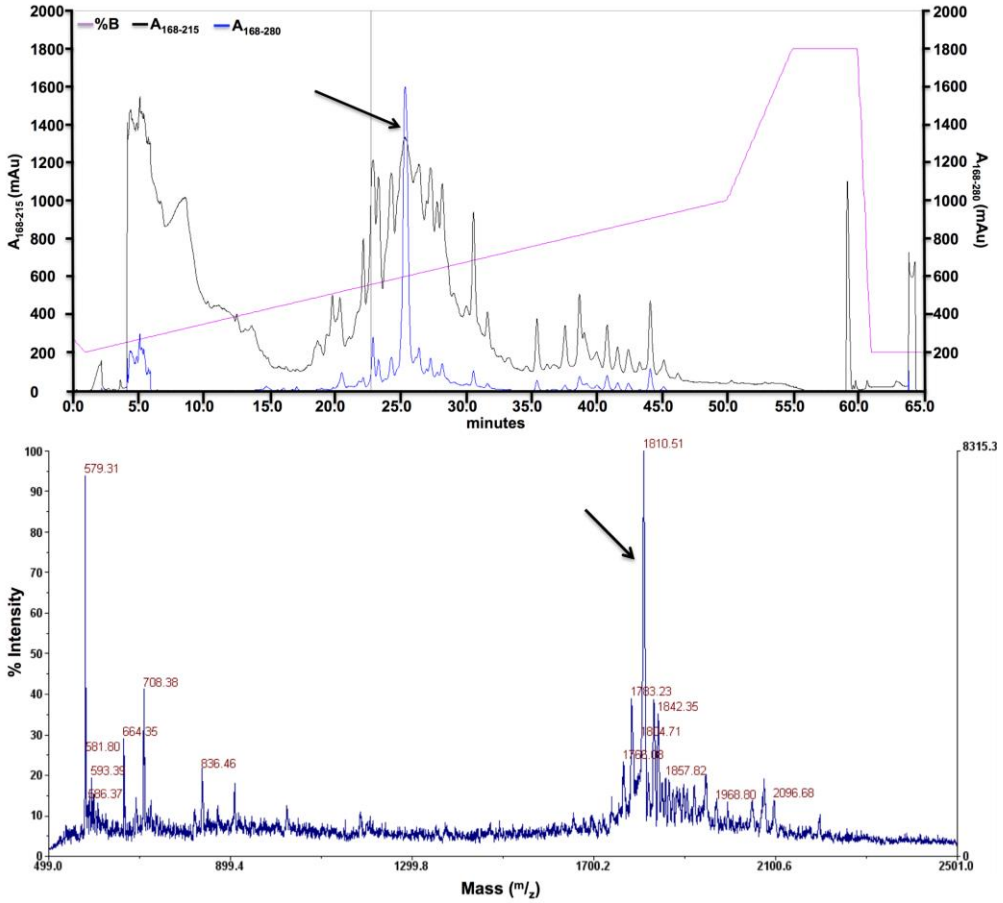


Figure A2B.11. The characterization data for scrambled SynEp2. Scrambled SynEp2 [I→$^{4\text{-}N3}$K]LSTGEYDAQE-PEG$_5$-Biotin: $C_{77}H_{124}N_{20}O_{29}S$. (Top) The semi-preparative HPLC trace for scrambled SynEp2 with the peak containing the correct mass indicated by an arrow. (Bottom) The MALDI-TOF spectra for scrambled SynEp2 with the indicated peak corresponding to the epitope with a single aspartamide formation [M-OH+H], expected [M-OH+H]=1809.99 amu.

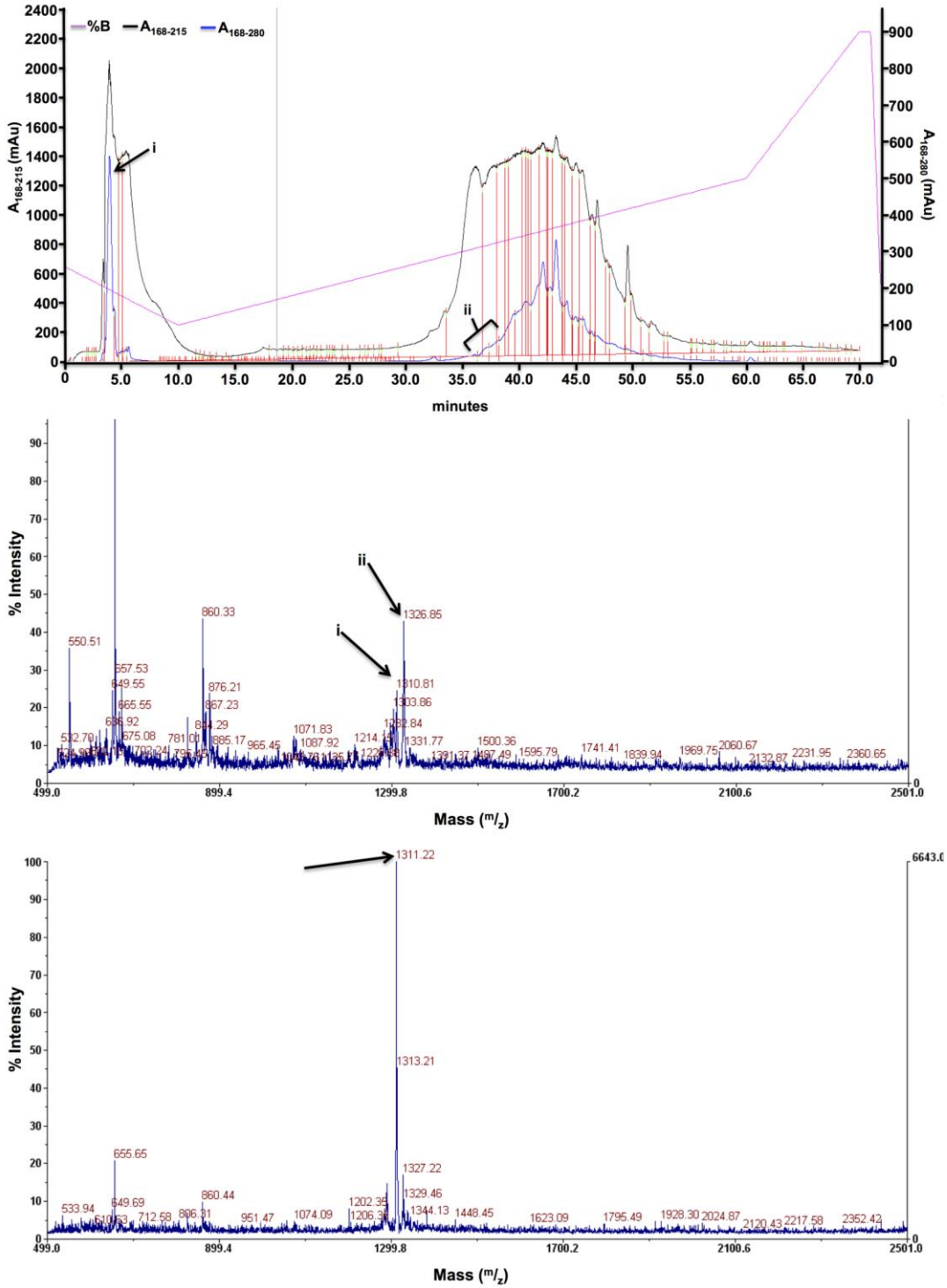


Figure A2B.12. **The characterization data for Ligand L1.** Ligand **L1** (Cyclic)-Pra-HGIVG-$^{4\text{-}N3}$K-PEG_5-biotin: $C_{57}H_{95}N_{17}O_{15}S$. (Top) The semi-preparative HPLC trace for Ligand L1. The peak labeled "i" corresponds to fraction L1a, and the peak labeled "ii" corresponds to fraction L1b. (Middle) The MALDI-TOF spectra for ligand fraction L1a. The indicated peak labeled "i" corresponds to the [M+H], expected [M+H] = 1290.699 amu, and the peak labeled "ii" corresponds to [M+Na], expected [M+Na] = 1312.661 amu. (Bottom) The MALDI-TOF spectra for ligand fraction L1b. The indicated peak corresponds to [M+Na], expected [M+Na] = 1312.661 amu.

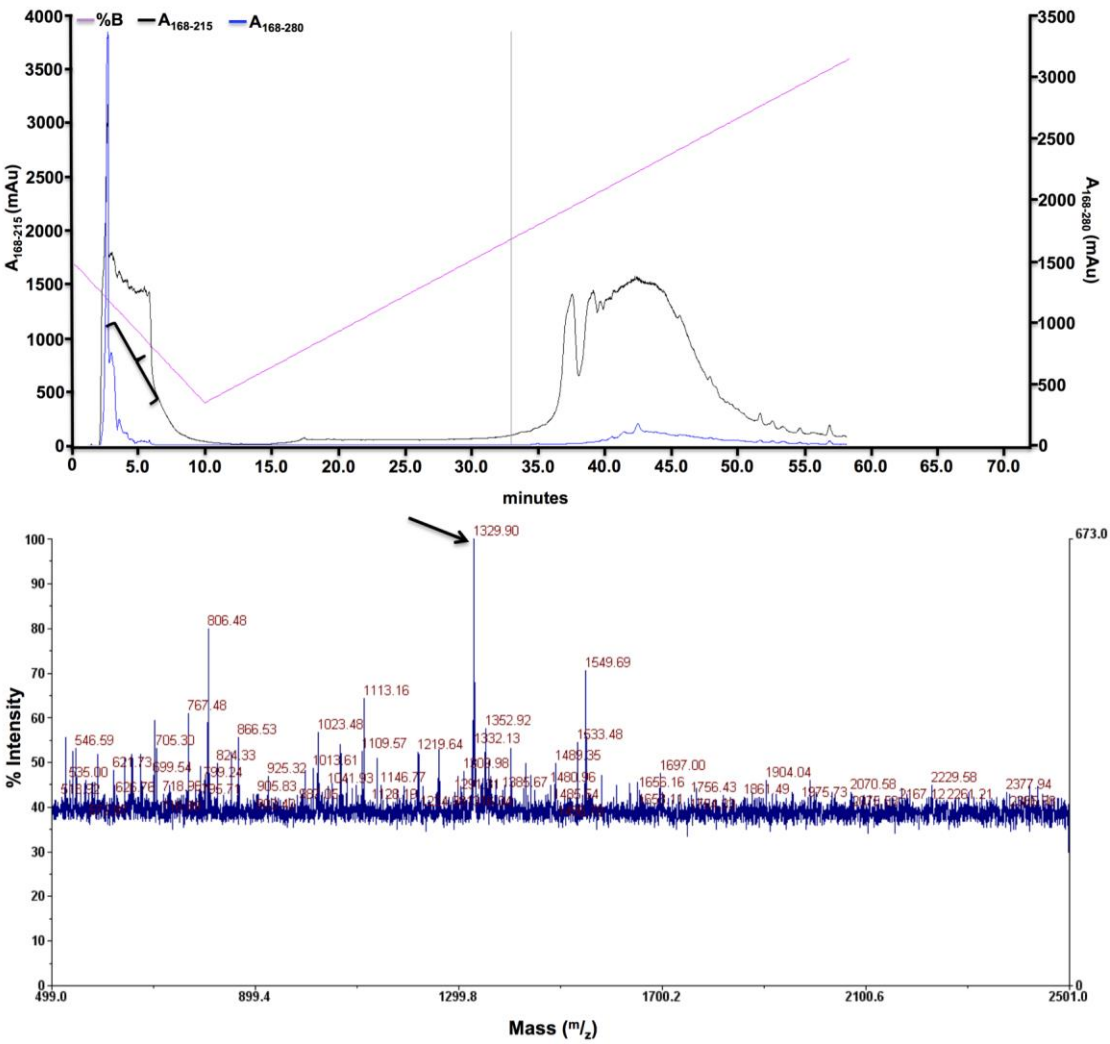


Figure A2B.13. **The characterization data for Ligand L2.** Ligand **L2** (Cyclic)-Pra-EGVPV-$^{4\text{-}N3}$K-PEG_5-biotin: $C_{58}H_{97}N_{15}O_{17}S$. (Top) The semi-preparative HPLC trace for Ligand L2. The peak labeled with an arrow corresponds to the peak with the correct mass. (Bottom) The MALDI-TOF spectra for ligand L2. The indicated peak corresponds to [M+Na], expected [M+Na] = 1330.682 amu.

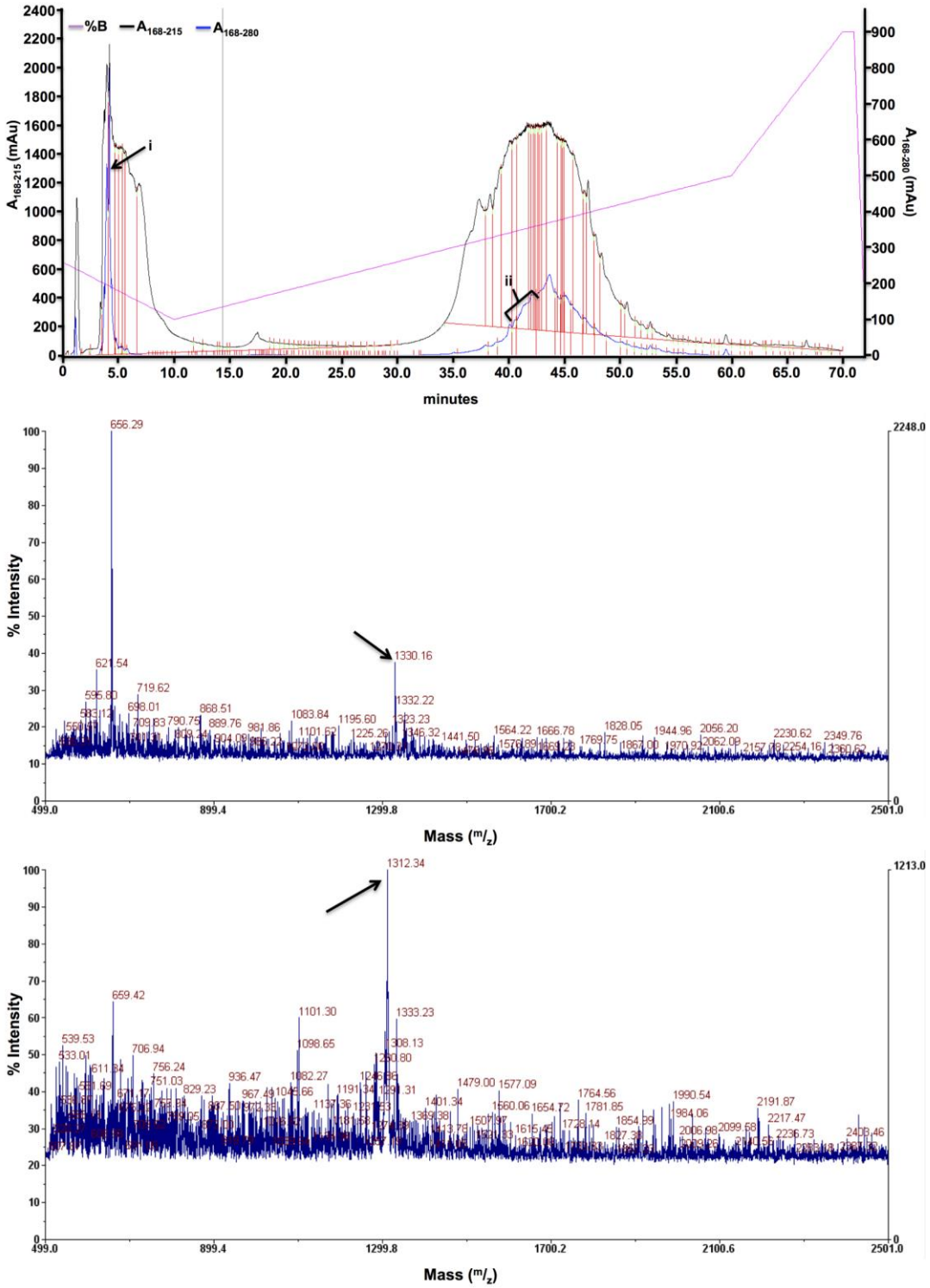


Figure A2B.14. The chatacterization data for Ligand L3. Ligand **L3** (Cyclic)-Pra-GEVVP-$^{4\text{-}N3}$K-PEG_5-biotin: $C_{58}H_{97}N_{15}O_{17}S$. (Top) The semi-preparative HPLC trace for Ligand L3. The peak labeled "i" corresponds to fraction L3a, and the peak labeled "ii" corresponds to fraction L3b. (Middle) The MALDI-TOF spectra for ligand fraction L3a. The indicated peak corresponds to the [M+Na], expected [M+Na] = 1330.682 amu. (Bottom) The MALDI-TOF spectra for ligand fraction L3b. The indicated peak corresponds to [M+H], expected [M+H] = 1308.699 amu.

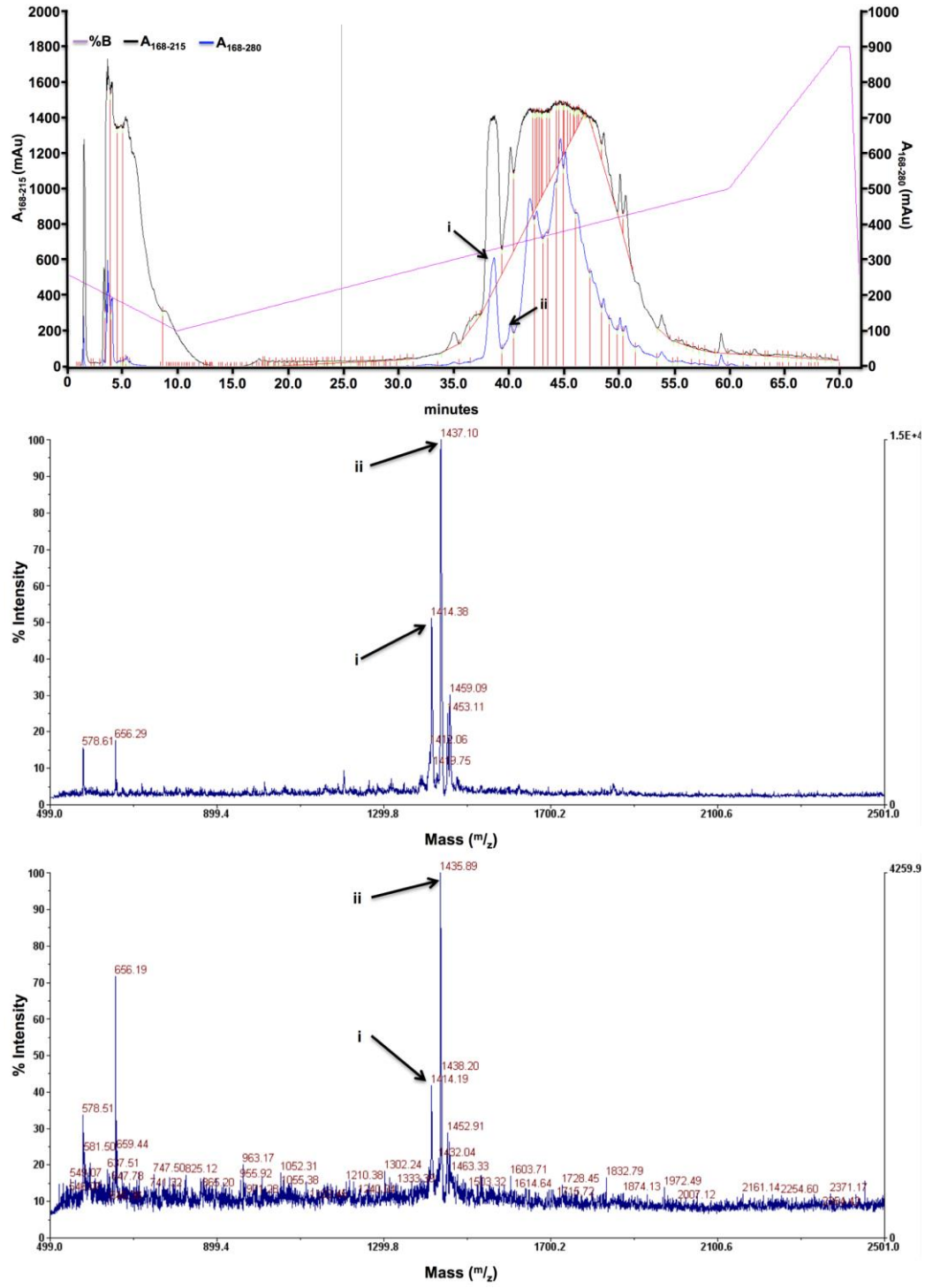


Figure A2B.15. The characterization data for Ligand L4. Ligand **L4** **(**Cyclic)-Pra-VEVPY-$^{4\text{-}N3}$K-PEG_5-biotin: $C_{65}H_{103}N_{15}O_{18}S$. (Top) The semi-preparative HPLC trace for Ligand L4. The peak labeled "i" corresponds to fraction L4a, and the peak labeled "ii" corresponds to fraction L4b. (Middle) The MALDI-TOF spectra for ligand fraction L4a. The indicated peak labeled "i" corresponds to the [M+H], expected [M+H] = 1414.740 amu, and the peak labeled "ii" corresponds to [M+Na], expected [M+Na] = 1436.722 amu. (Bottom) The MALDI-TOF spectra for ligand fraction L4b. The indicated peak labeled "i" corresponds to the [M+H], and the peak labeled "ii" corresponds to [M+Na].

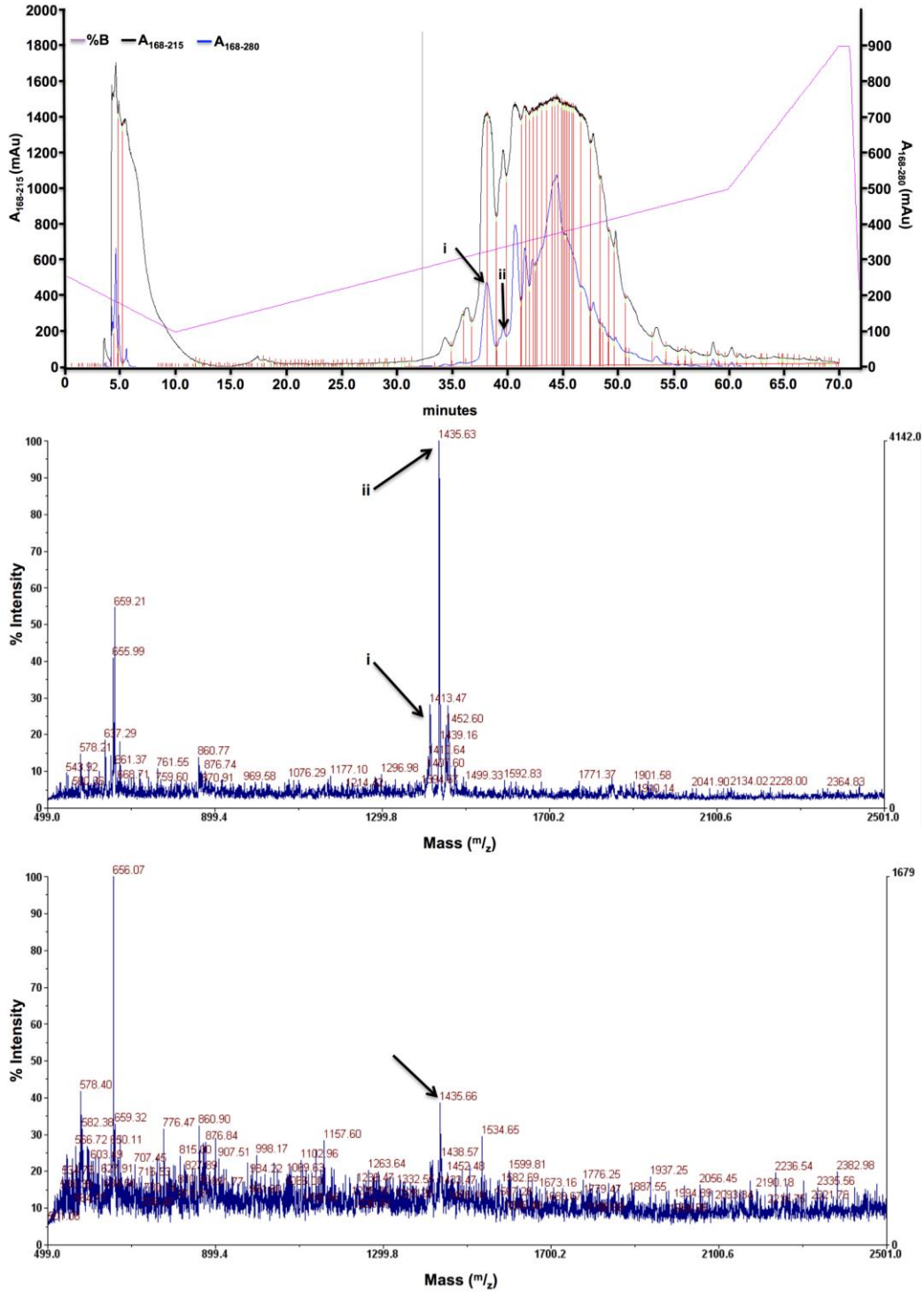


Figure A2B.16. Characterization data for Ligand L5. Ligand **L5** (Cyclic)-Pra-VEVYP-$^{4\text{-}N3}$K-PEG$_5$-biotin: $C_{65}H_{103}N_{15}O_{18}S$. (Top) The semi-preparative HPLC trace for Ligand L5. The peak labeled "i" corresponds to fraction L5a, and the peak labeled "ii" corresponds to fraction L5b. (Middle) The MALDI-TOF spectra for ligand fraction L5a. The indicated peak labeled "i" corresponds to the [M+H], expected [M+H] = 1414.740 amu, and the peak labeled "ii" corresponds to [M+Na], expected [M+Na] = 1436.722 amu. (Bottom) The MALDI-TOF spectra for ligand fraction L4b. The indicated peak corresponds to [M+Na].

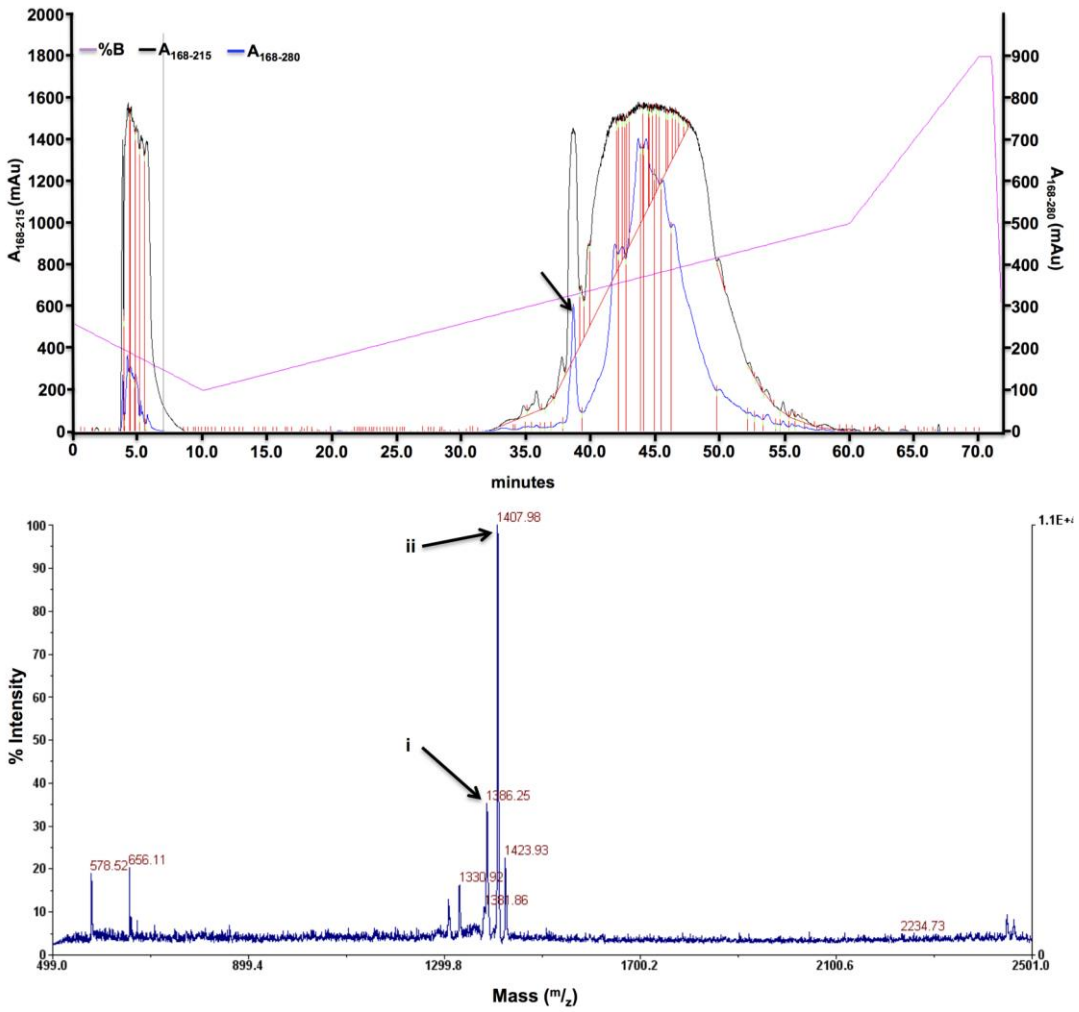


Figure A2B.17. The characterization data for Ligand L6. Ligand **L6** (Cyclic)-Pra-VTVPY-$^{4\text{-}N3}$K-PEG_5-biotin: $C_{64}H_{103}N_{15}O_{17}S$. (Top) The semi-preparative HPLC trace for Ligand **L6**. The peak labeled with an arrow corresponds to the peak with the correct mass. (Bottom) The MALDI-TOF spectra for ligand **L6**. The indicated peak labeled "i" corresponds to [M+H], expected [M+H] = 1386.746, and the peak labeled "ii" corresponds to [M+Na], expected [M+Na] = 1408.727 amu.

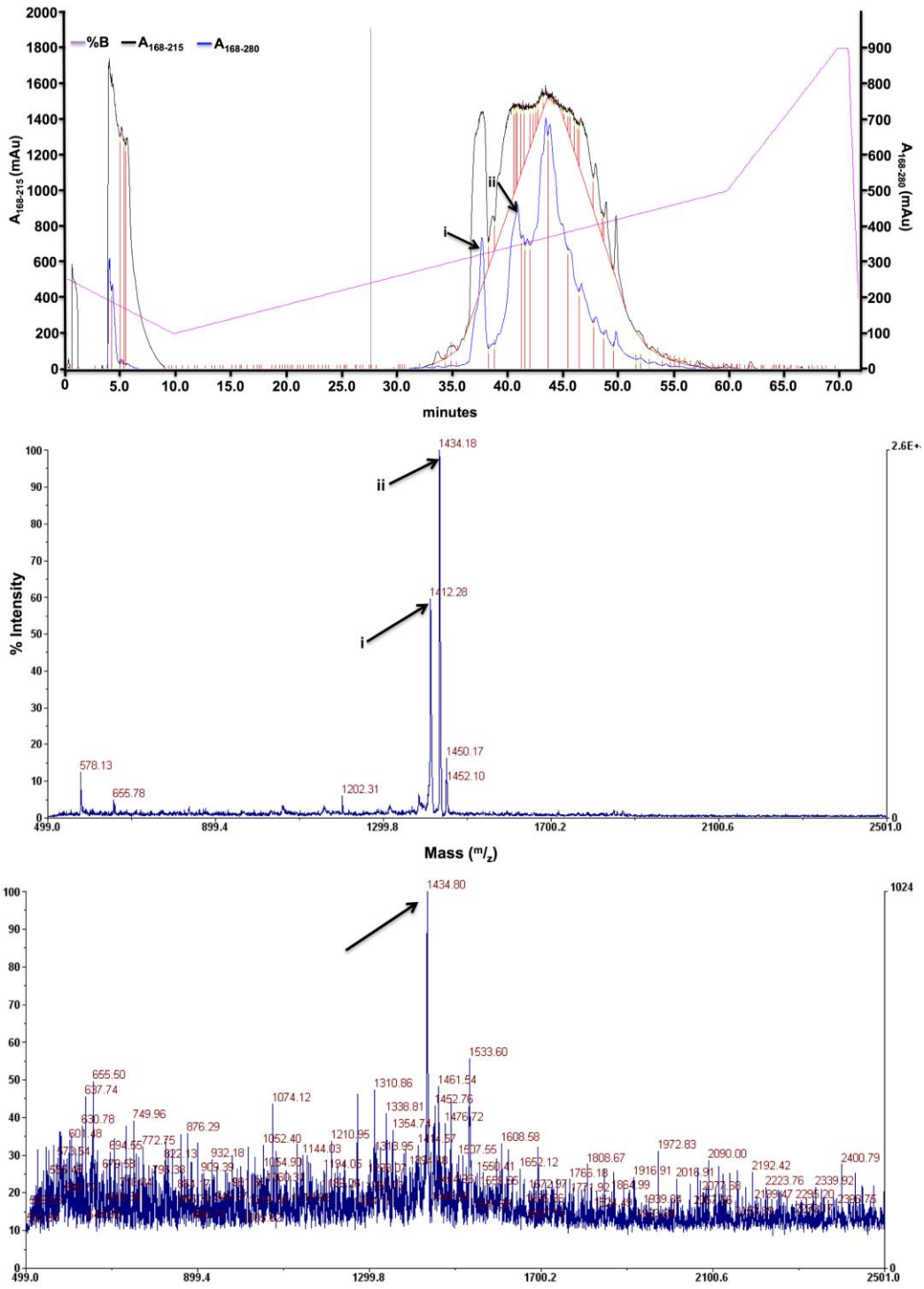


Figure A2B.18. Characterization data for Ligand L7. Ligand **L7** (Cyclic)-Pra-VQVPY-$^{4\text{-}N3}$K-PEG$_5$-biotin: $C_{65}H_{104}N_{16}O_{17}S$. (Top) The semi-preparative HPLC trace for Ligand **L7**. The peak labeled "i" corresponds to fraction **L7a**, and the peak labeled "ii" corresponds to fraction **L7b**. (Middle) The MALDI-TOF spectra for ligand fraction **L7a**. The indicated peak labeled "i" corresponds to the [M+H], expected [M+H] = 1413.722 amu, and the peak labeled "ii" corresponds to [M+Na], expected [M+Na] = 1435.738 amu. (Bottom) The MALDI-TOF spectra for ligand fraction **L7b**. The indicated peak corresponds to [M+Na].

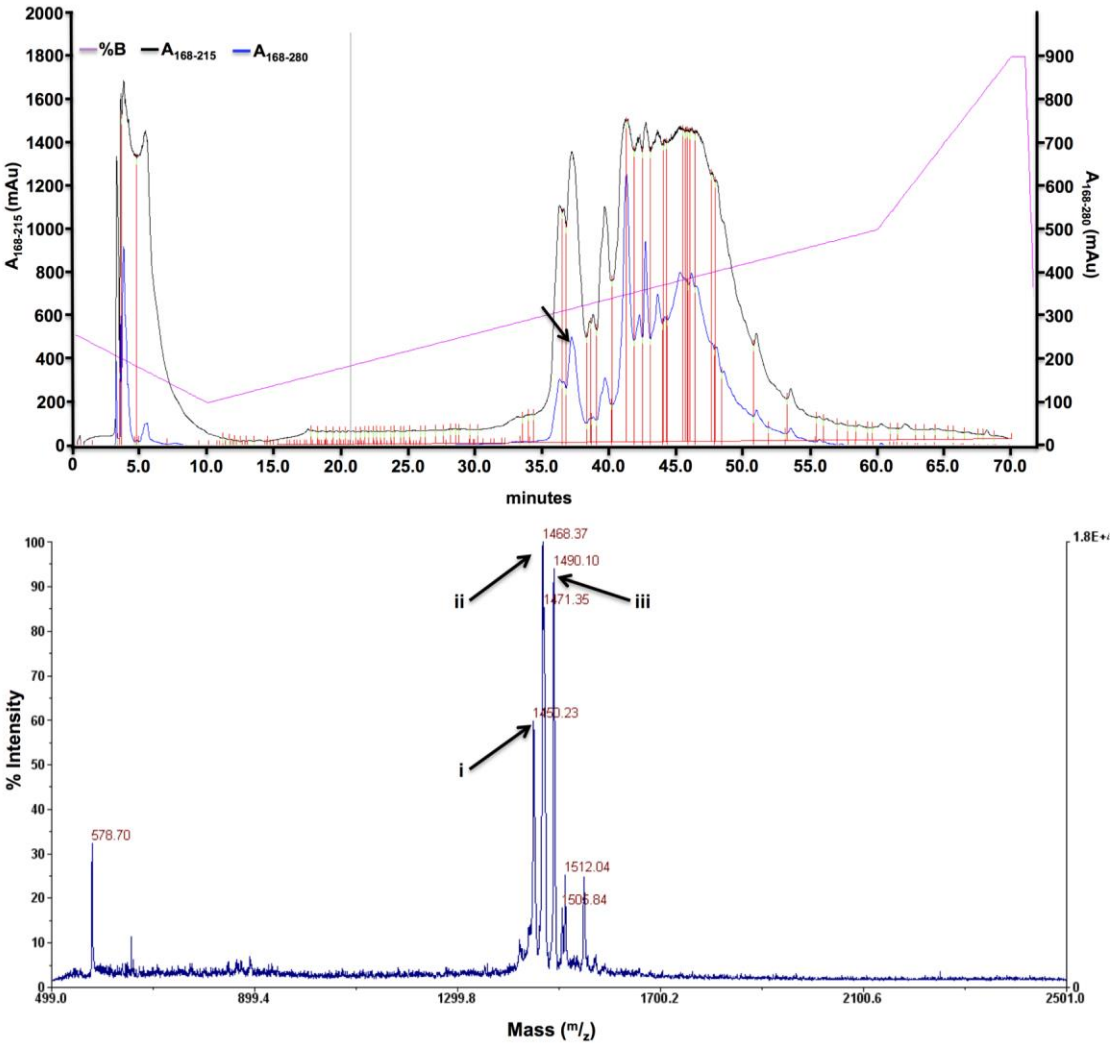


Figure A2B.19. The characterization data for Ligand L8. Ligand **L8** (Cyclic)-Pra-TDNFY-$^{4\text{-}N3}$K-PEG_5-biotin: $C_{66}H_{98}N_{16}O_{20}S$. (Top) The semi-preparative HPLC trace for Ligand **L8**. The peak labeled with an arrow corresponds to the peak with the correct mass. (Bottom) The MALDI-TOF spectra for ligand **L8**. The indicated peak labeled "i" corresponds to aspartamide formation [M-OH+H], expected [M-OH+H] = 1450.691 amu, the peak labeled "ii" corresponds to [M+H], expected [M+H] = 1467.694 amu, and the peak labeled "iii" corresponds to [M+Na], expected [M+Na] = 1489.676 amu.

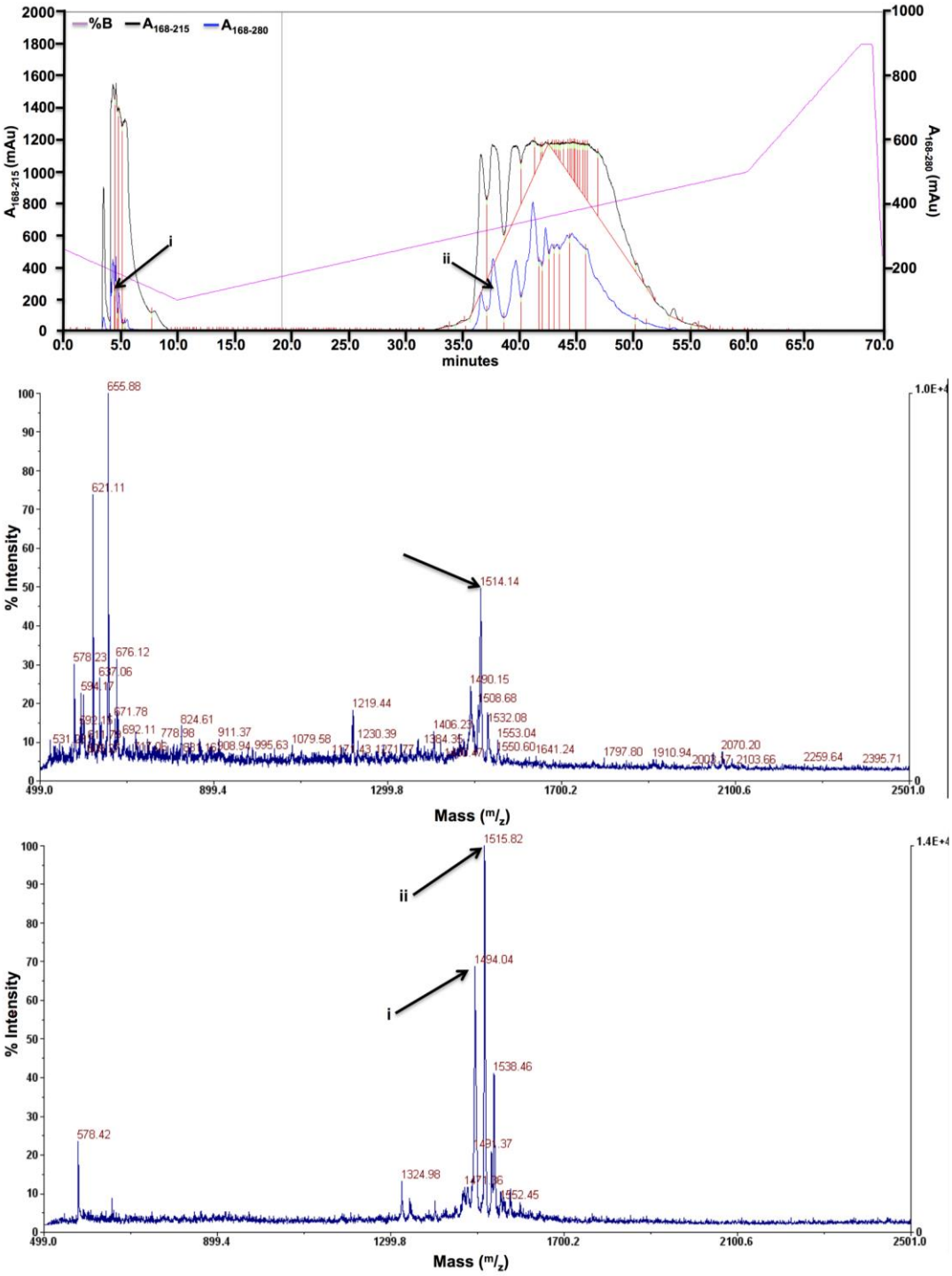


Figure A2B.20. The characterization data for ligand L9. Ligand **L9** (Cyclic)-Pra-QDNFY-$^{4\text{-}N3}$K-PEG_5-biotin: $C_{67}H_{99}N_{17}O_{20}S$. (Top) The semi-preparative HPLC trace for Ligand L9. The peak labeled "i" corresponds to fraction L9a, and the peak labeled "ii" corresponds to fraction L9b. (Middle) The MALDI-TOF spectra for ligand fraction L9a. The indicated peak corresponds to [M+Na], expected [M+Na] = 1516.687 amu. (Bottom) The MALDI-TOF spectra for ligand fraction L9b. The indicated peak labeled "i" corresponds to the [M+H], expected [M+H] = 1494.705 amu, and the peak labeled "ii" corresponds to [M+Na], expected [M+Na] = 1516.687 amu.

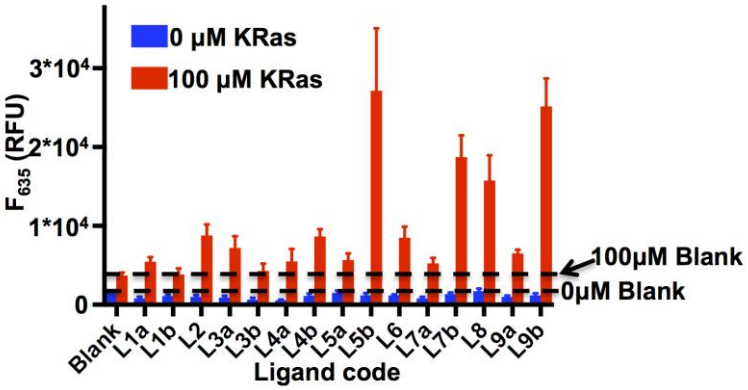


Figure A2B.21. Single point Immunofluorescent Assay using the B-RAP technology. A single point Immunofluorescent assay on the rapid assay platform for the KRas allosteric switch ligands.

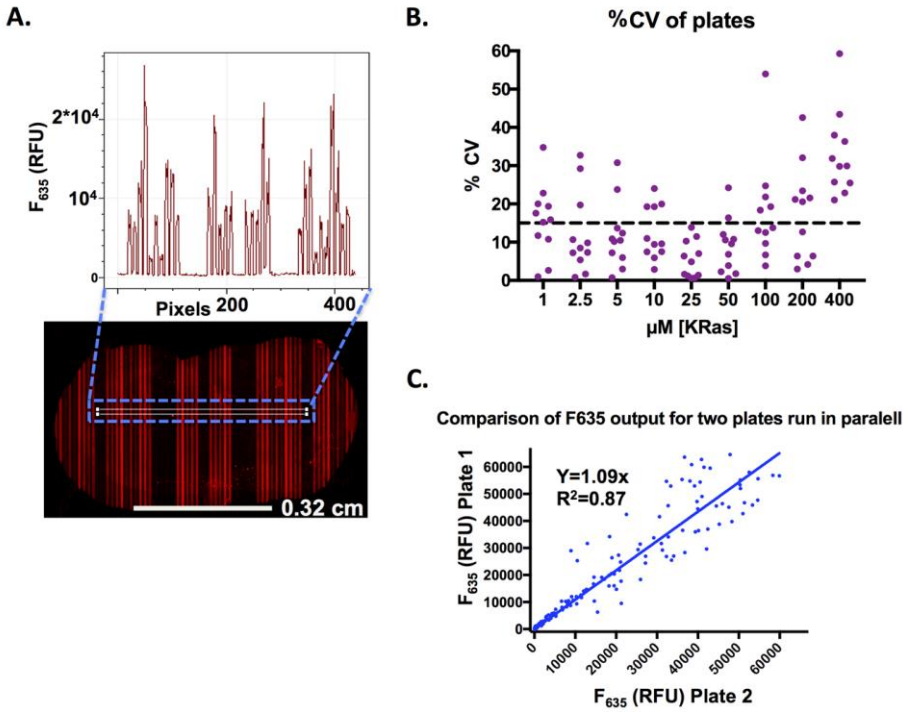


Figure A2B.22. Statistical validation of the B-RAP Technology. A. Comparing the two full sets of DNA barcode in a given microwell at 5μM KRas reveals that extracting data from a single barcode repeat is sufficient. B. The %CV for 1 μM to 400 μM KRas with the average %CV for the entire concentration range (~18%) indicated by the black dashed line. C. A plot of the F_{635} values for both plates plotted against each other. The good correlation at low F_{635} values indicates that the variance at low [KRas protein] is satisfactory.

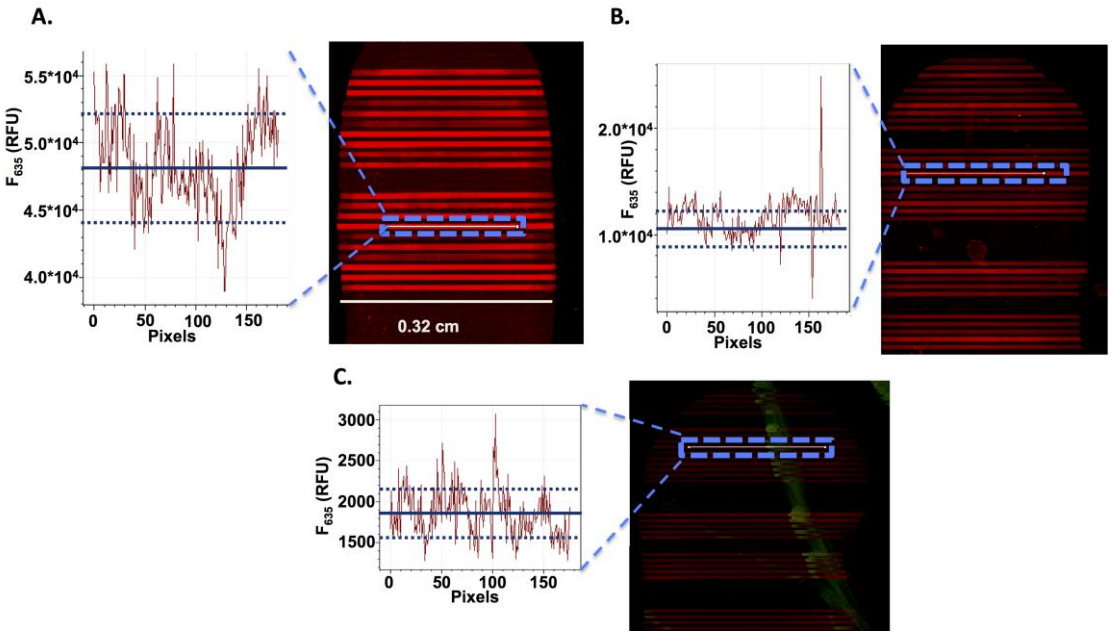


Figure A2B.23. A comparison of the average line-scan with an averaged data-block extraction. A comparison of the full-line scan with the average F_{635} resulting from averaging ten discrete data block extractions and the corresponding standard deviations at A. 10 μM KRas protein: avgF_{635}=47,868.7, std-dev=4154.53. B. 1 μM KRas protein: avgF_{635}=11437.6, std-dev=1602.202. C. 100 nM KRas Protein, avgF_{635}=1780.6, std-dev=236.38.

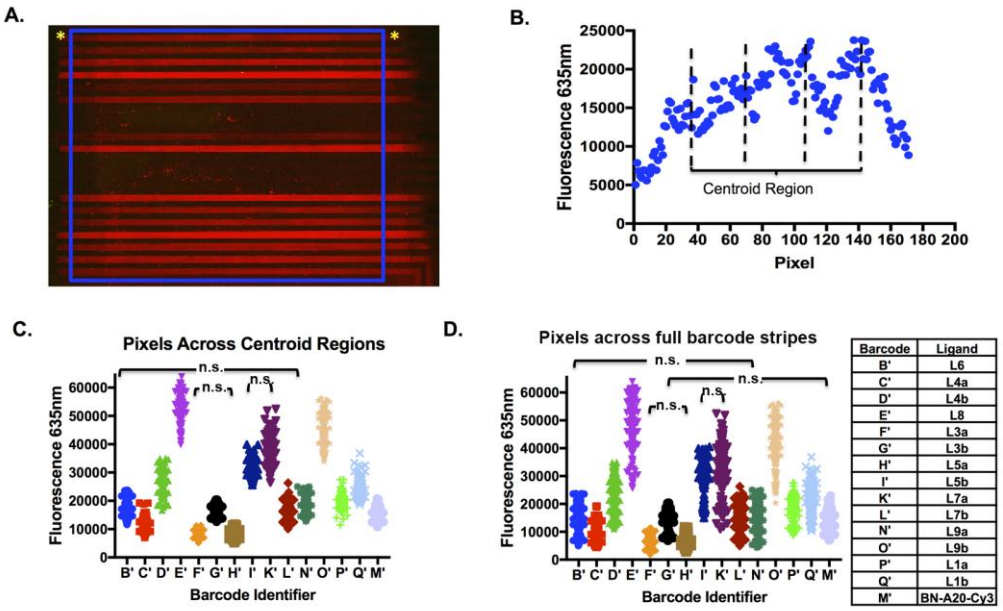
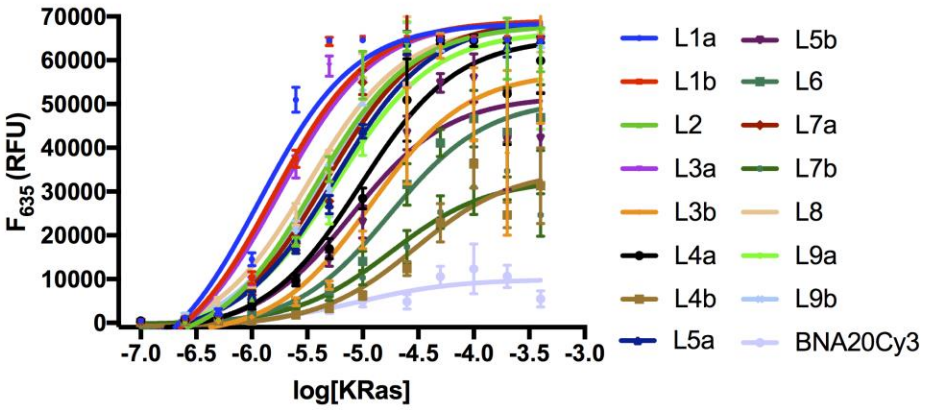


Figure A2B.24. Pixel extraction of full barcode lanes in a given barcode. A. The region of the barcode lanes extracted with the B' lane graphed in a) set off by yellow asterisks. B. A close up of the scatterplot of barcode lane B' to highlight where the centroid region was defined and extracted (pixel 40-142) with the region divided into thirds for the left, middle, and right centroid respectively C. A scatterplot of the F635 extracted for individual pixels along the entire length of a barcode in the 10 μM well of plate #1 with lanes that were indistinguishable by a two-tailed student T test indicated by brackets, and an accompanying key for parts c) and d). D. A scatterplot of the F_{635} extracted for individual pixels in the centroid region of each barcode with lanes that were indistinguishable by a two-tailed student T-test (Table S5) indicated by brackets.

A.



B.

Ligand	L1a	L1b	L2	L3a	L3b	L4a	L4b	L5a	L5b	L6[a]	L7a	L7b	L8	L9a	L9b	Blank
EC_{50} (μM)	1.16± 0.20	1.56± 0.26	3.7±0. 53	1.75± 0.25	11.86 ±4.0	9.51± 1.9	29.80 ±8.7	5.63± 0.57	8.04± 2.2	17.62 ±3.5	4.63± 0.64	18.00 ±5.0	3.22± 0.39	5.84± 1.0	4.31± 0.62	9.49± 5.6

Figure A2B.25. KRas binding curves without dummy ligand extraction. A. The KRas protein binding curves for the candidate PCC ligands run without the dummy ligand (BN-A_{20}-Cy3) correction. B. The EC_{50} values for L1a to L9b. [a]Ligand **L6** was run separately.

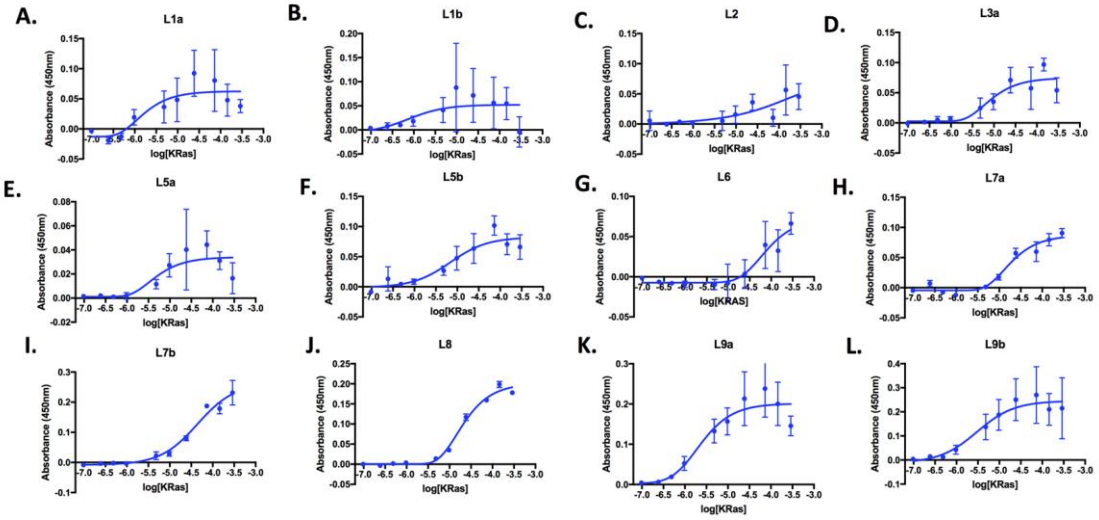


Figure A2B.26. KRas protein binding curves using 96-well plate ELISAs.

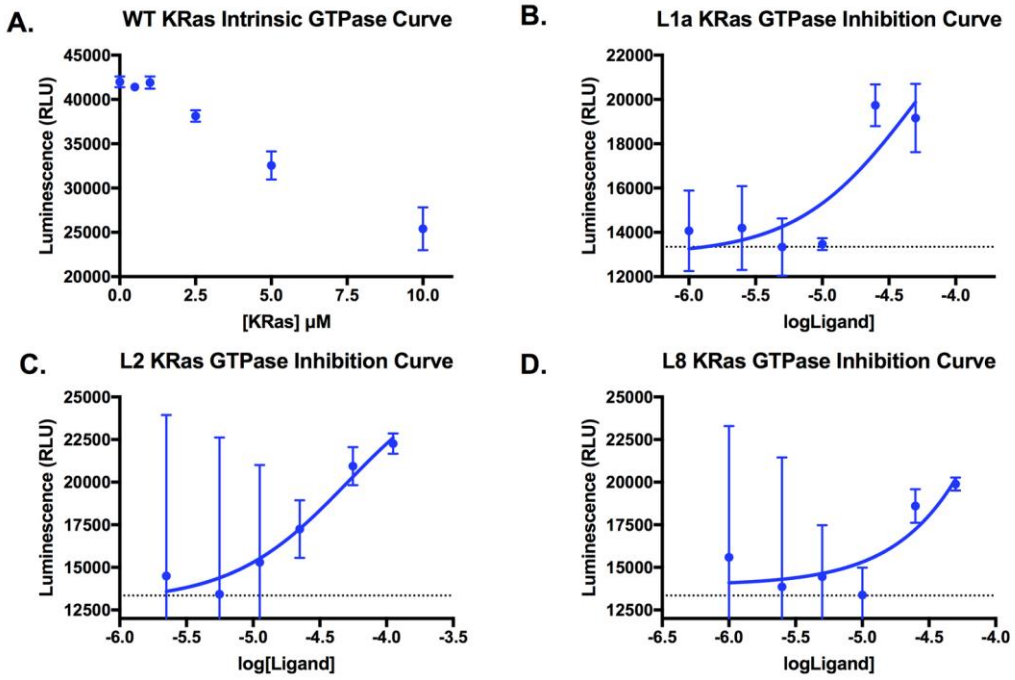


Figure A2B.27. The wild-type KRas protein GTPase activity curves. A. The WT Kras standard curve generated with a one hour incubation with 5 µM rGTP. The inhibition curves generated for the best three ligand fractions with a two hour rGTP incubation period in B., C., and D. The dotted line represents the luminescence measurement (~13k RLU) when 10 µM KRas was incubated with 5 µM rGTP for two hours without ligand.

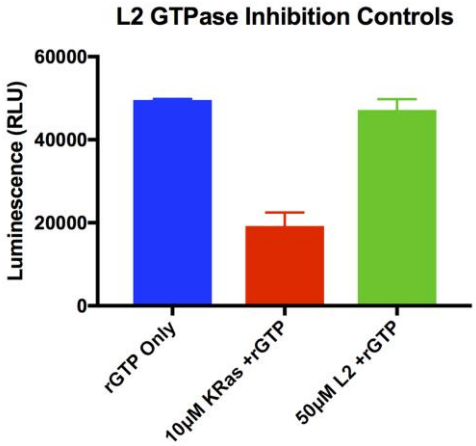


Figure A2B.28. Controls for the L2 GTPase inhibition curve. Controls run for the **L2** inhibition curve. It is apparent that ligand **L2** lacks GTPase catalytic activity as less than 5% of the GTP was hydrolyzed when placed with just 50 µM of **L2**.