## ***Combines Induction, Deduction & Direct Analysis for Exact Definition of Analyte.***

- Example: C-Peptide
  - FDA-Approved Test System for Abnormal Insulin Secretion *(21CFR862.1135)*
    - Sequence 0: EAEDLQVGQ*VELGGGPGAGSL*QPLALEGSLQ (*Epitope*)
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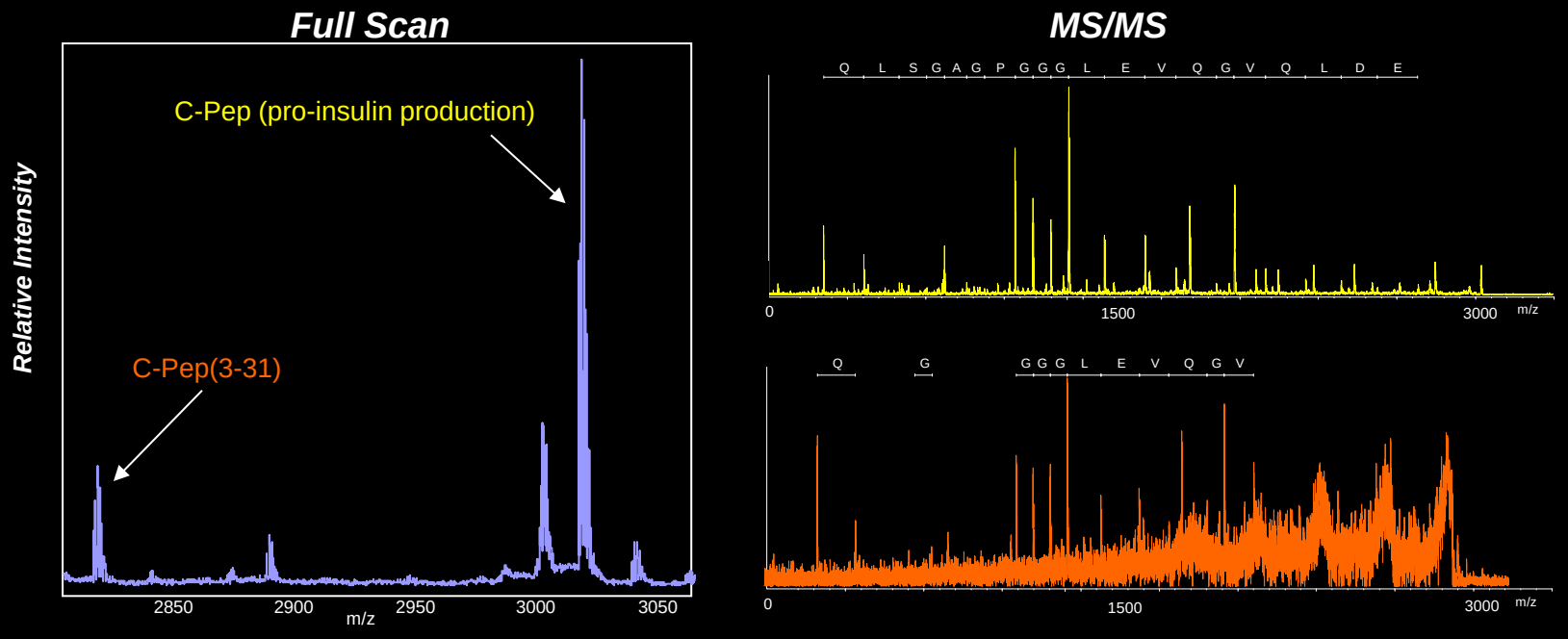

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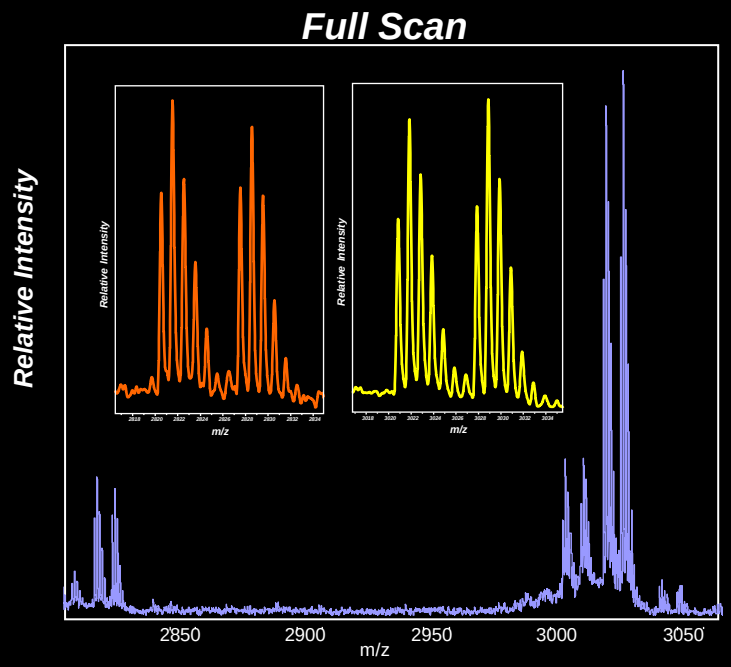
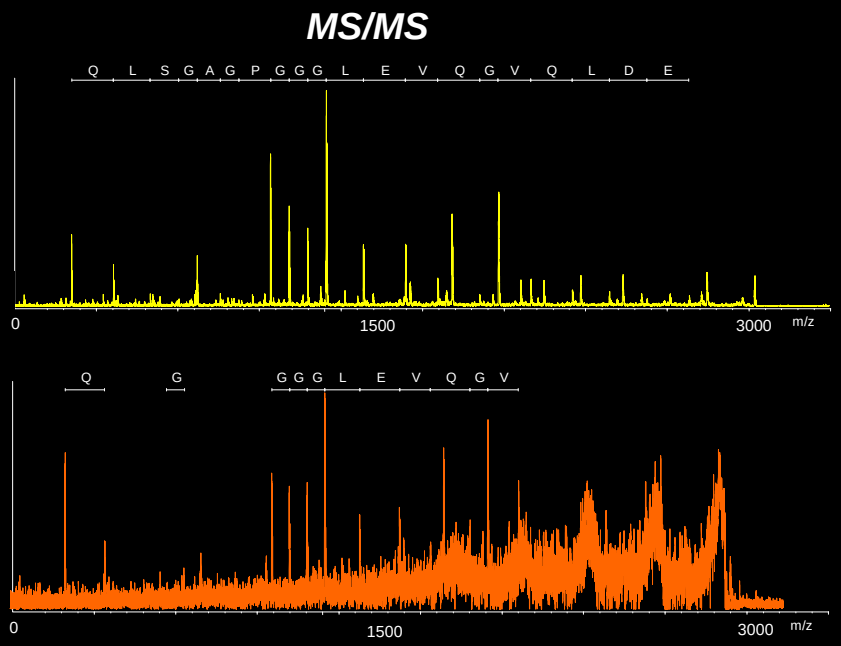
- Quantification:
  - Heavy isotopes
    - Added to biological samples and carried through entire MSIA process



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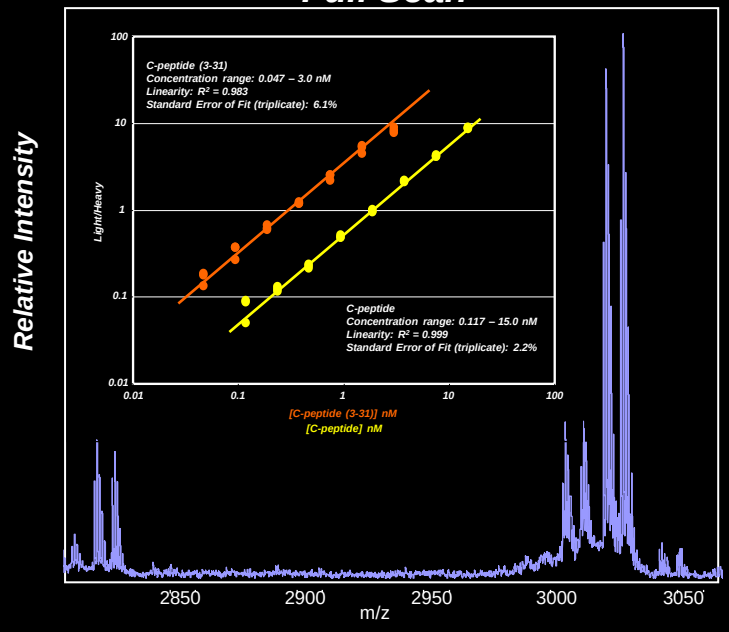
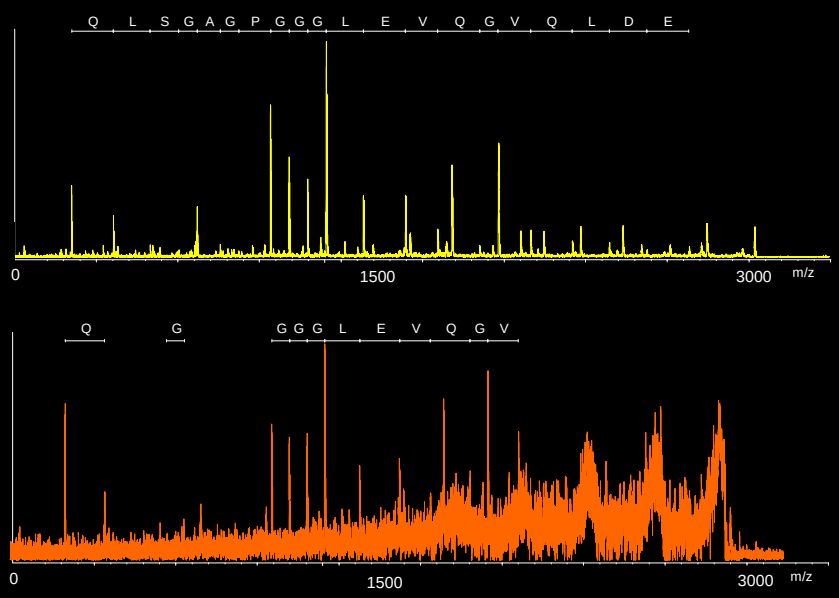
- Quantification:
  - Heavy isotopes
    - Added to biological samples and carried through entire MSIA process
  - Working curves generated simultaneously for both species
    - Linear Dynamic Range > 100; Std. Error < 6%; LOD's < 50 pM

***Full Scan***




***MS/MS***



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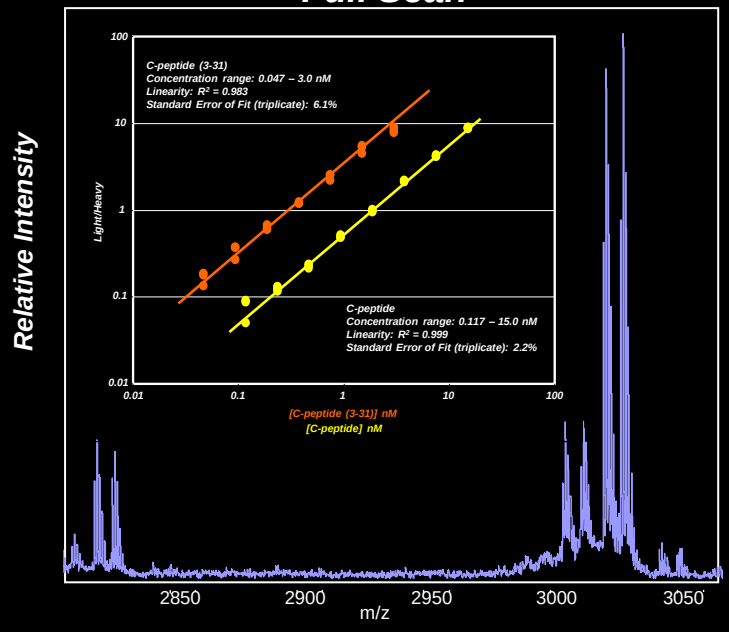
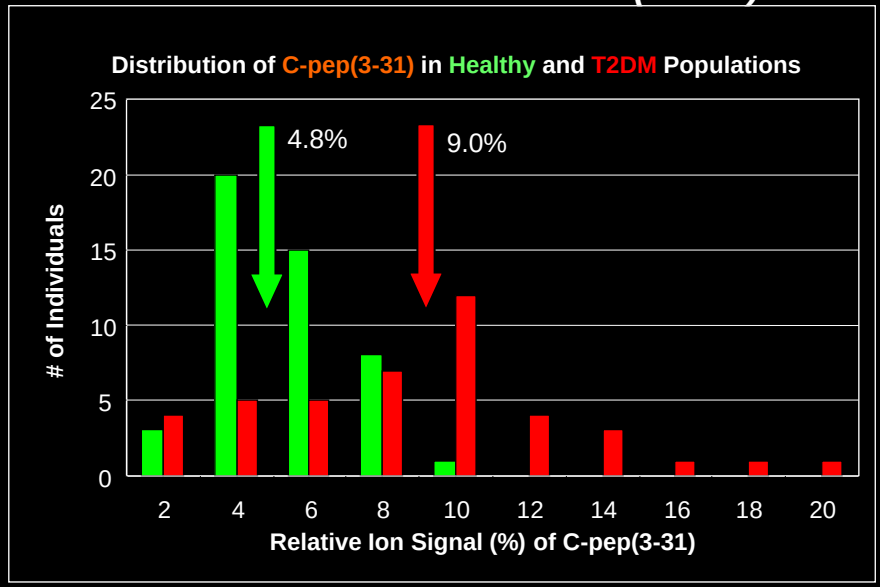
    - Sequence 2: ~~EA~~EDLQVGQVELGGGPGAGSLQPLALEGSLQ (Discover Variant)
- Implications in T2DM:
  - DPP-IV Motif – Target of T2DM DPP-IV Inhibitor Therapy *(Vildagliptin (e.g., Galvus))*
    - Variant Specific to Drug Target – Emphasizes the Stoichiometry between Variants
  - Quantitative Contribution of Variant can result in 100% Error in C-Peptide Determination
    - Population and/or Disease-Based Microheterogeneity

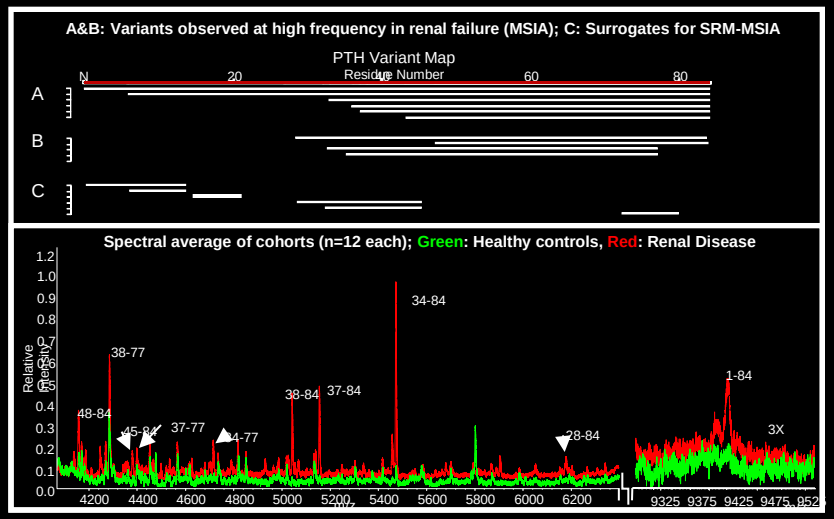
***Full Scan***

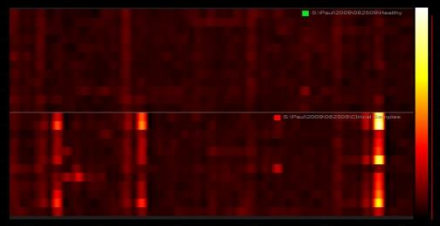


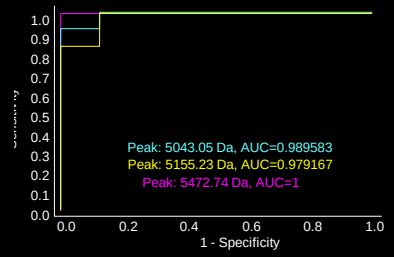

***Correlation in Disease (T2DM)***

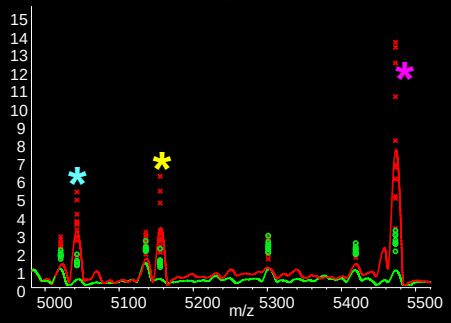


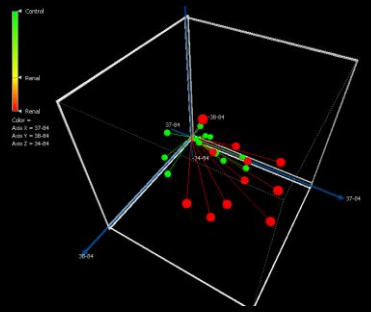
## MALDI-TOFMS



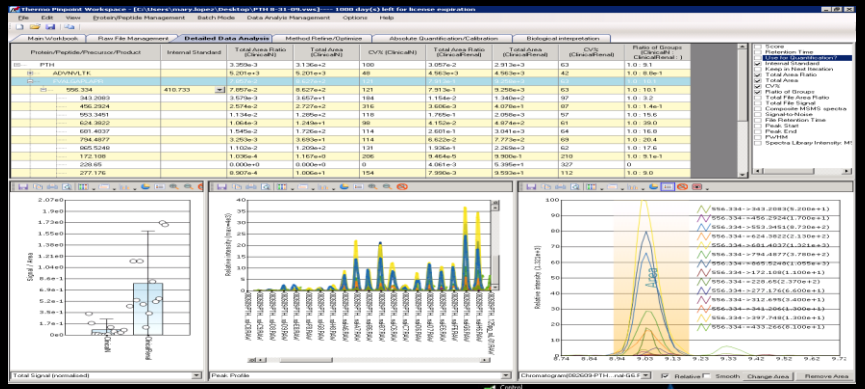



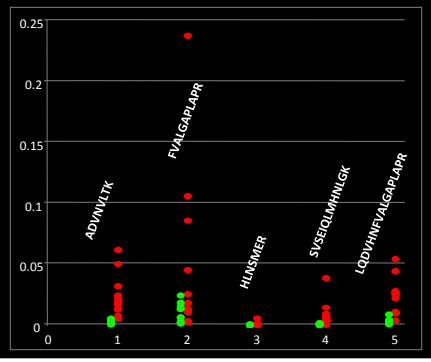


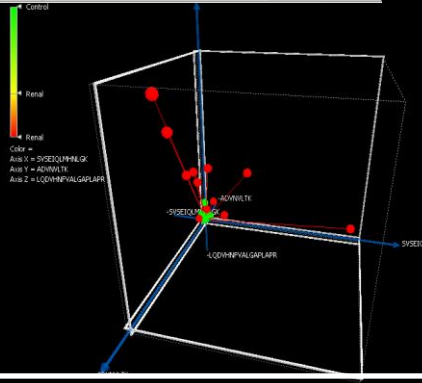




## ESI-SRM (Triple Quad)






- > 10 Variants (Known & Discovered)
  - Conversion to ESI-SRM-MSIA
    - Single Antibody/Post-extraction Digestion
    - 6 Variants; 32 SRM Transitions; > 50% Coverage
    - LOD < 10 pg/mL; LOQ 16 pg/mL; SE ~ 7%
  - Conversion to MALDI-MRM-MSIA
    - Bioreactive Plates
    - Full-scan & Tandem TOFMS

## ***Apolipoprotein A1***

**DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQEFWDNLEKETEGLRQE*M*SKDLEEVKAKVQPYLD**
**DFQKKWQEE*M*ELYRQKVEPLRAELQEGARQKLHELQEKLSPLGEE*M*RDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATEHLSTL**
**SEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTK*K*LNT*Q***

- ***Full-length Functional Form***

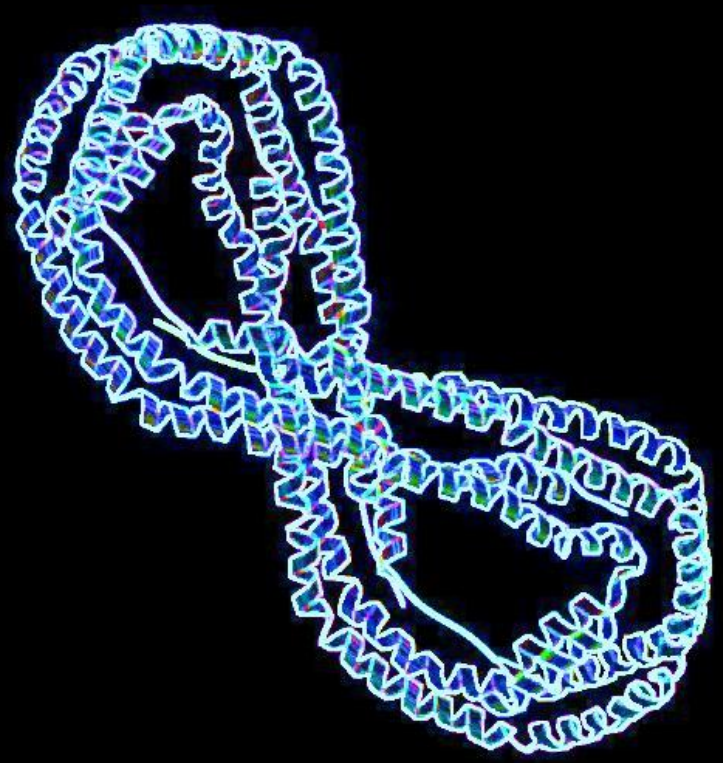
- MW = 28,080

- Reference:  106 – 220 mg/dL

- HDL Protein
  - Cholesterol Transport
  - Ratio relative to other Apolipoproteins

- Diseases
  - Heart diseases
  - Atherosclerosis
  - Tangiers

- Modified forms cause or created by disease
  - Oxidative Stress
  - Defense Mechanisms (Milano)
  - Environmental Effects
  - Hyperglycemia



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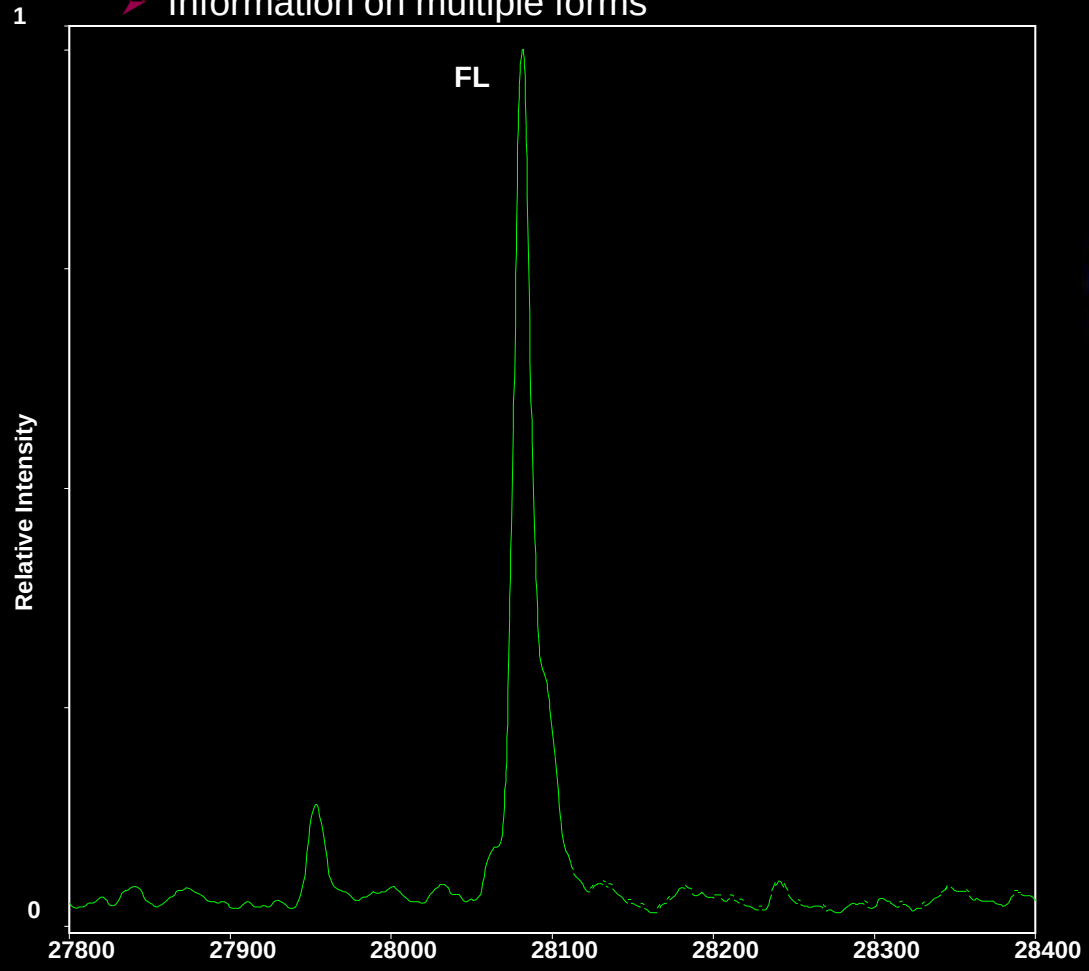
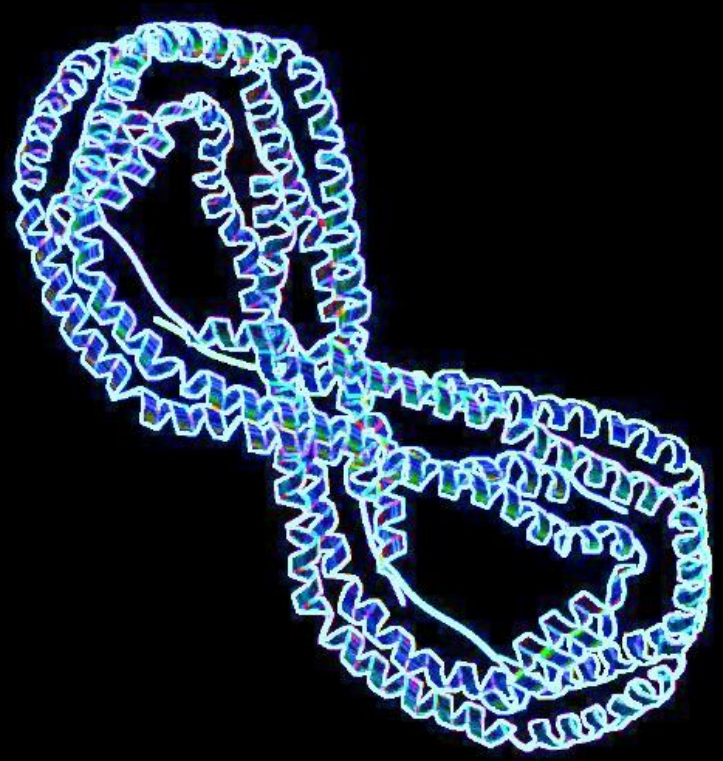
**SEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTK*K*LNT*Q***

- ***Full-length (Direct)***
  - Information on multiple forms



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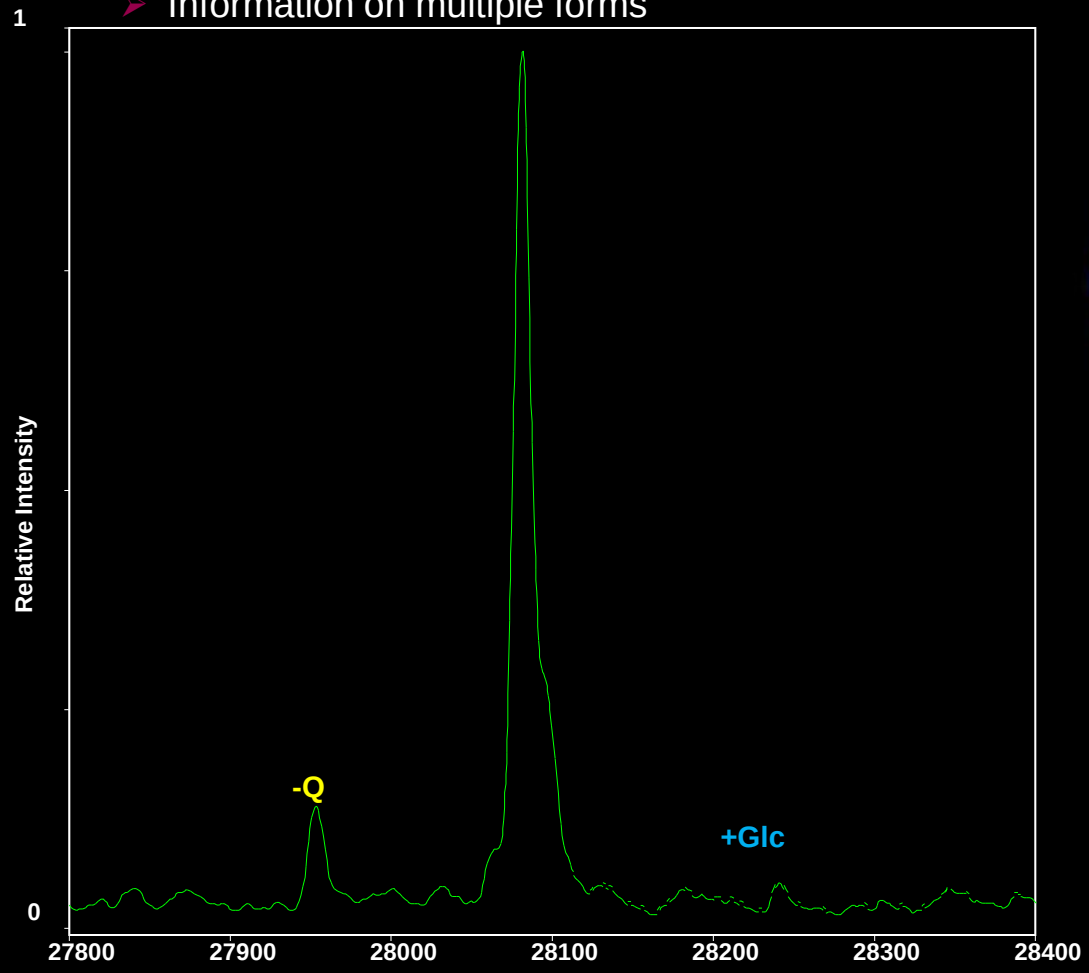
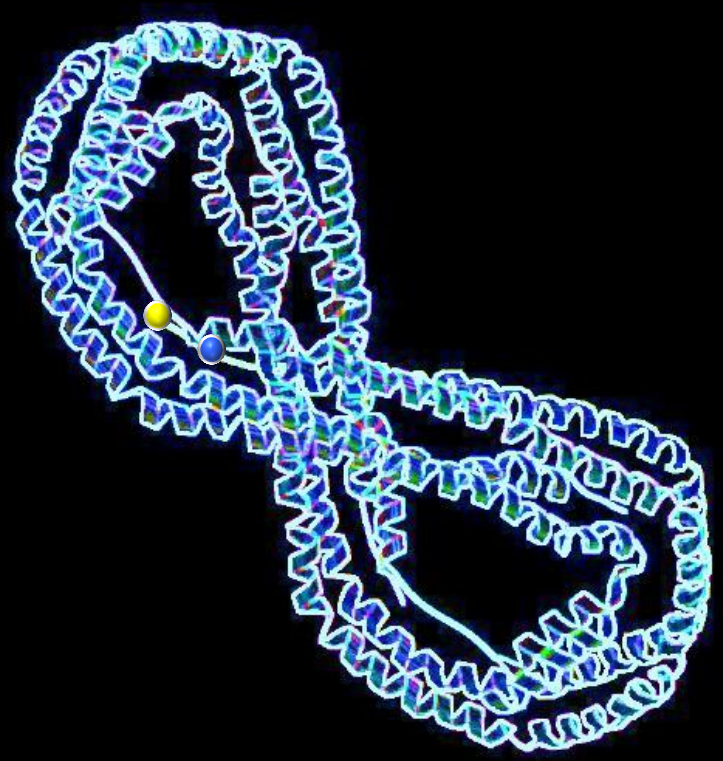
- ***Full-length (Direct)***
  - Information on multiple forms



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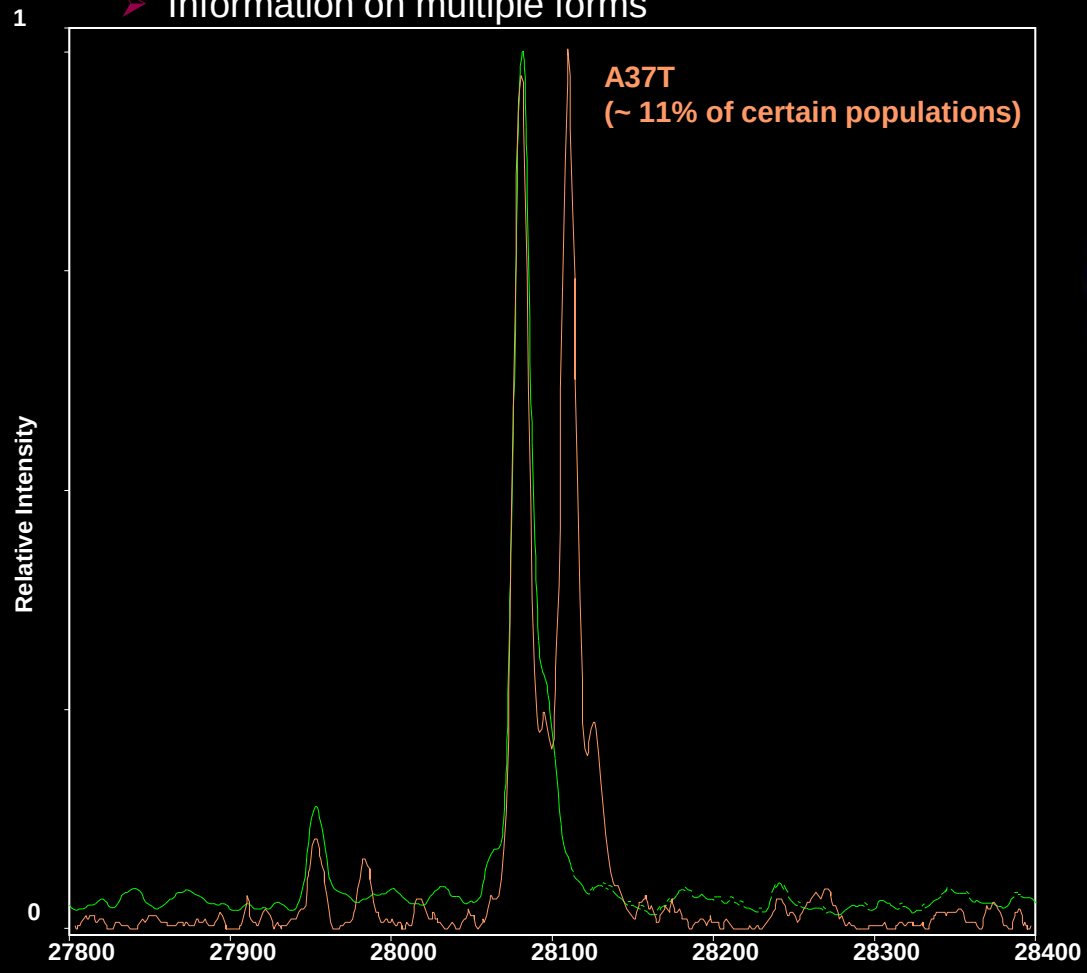
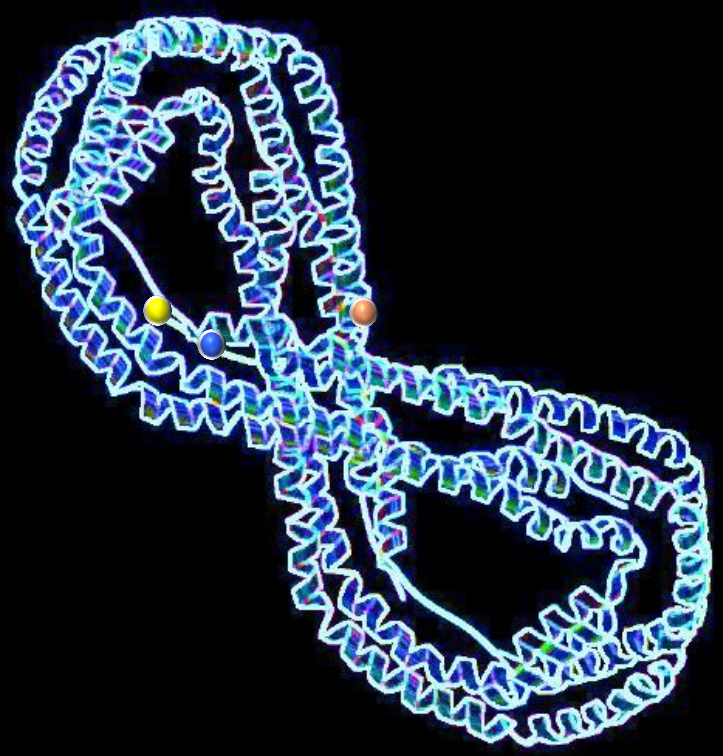
SEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTK*K*LNT*Q*

- ***Full-length (Direct)***
  - Information on multiple forms



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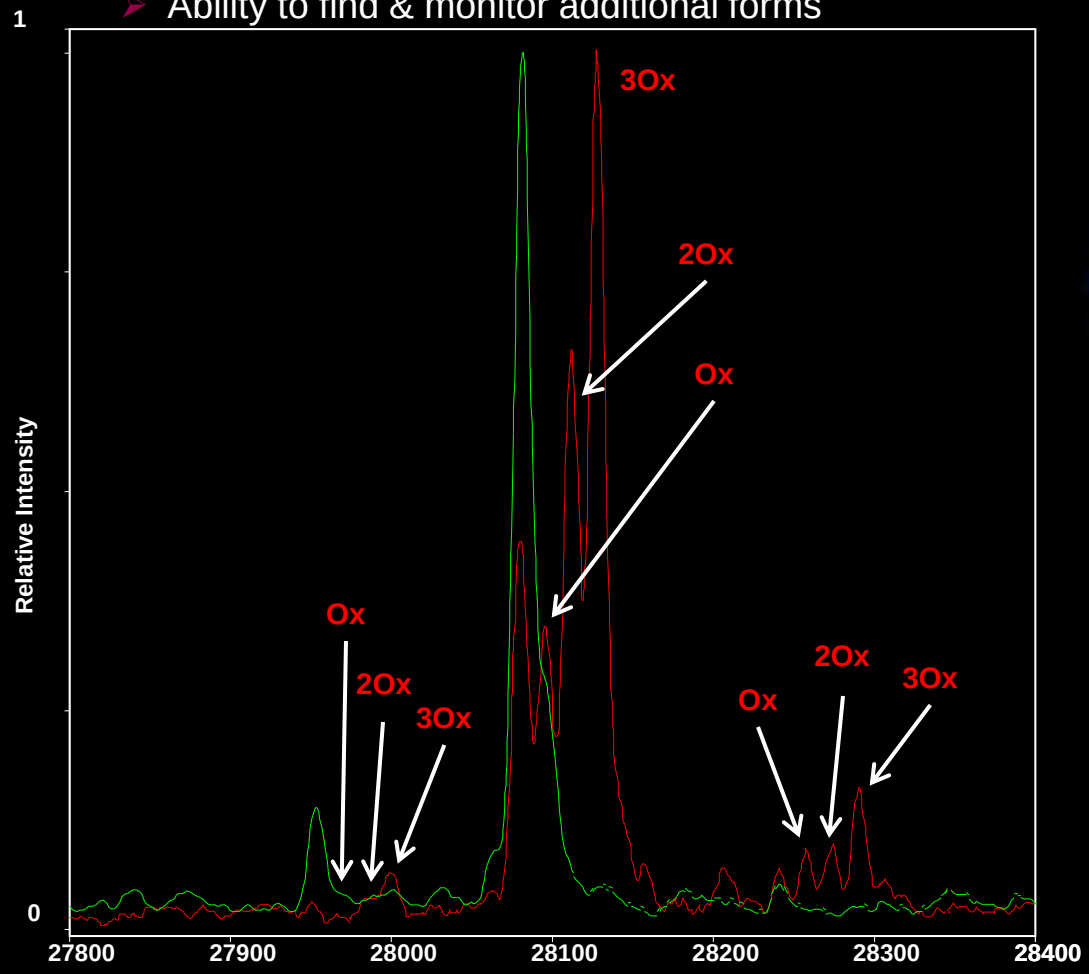
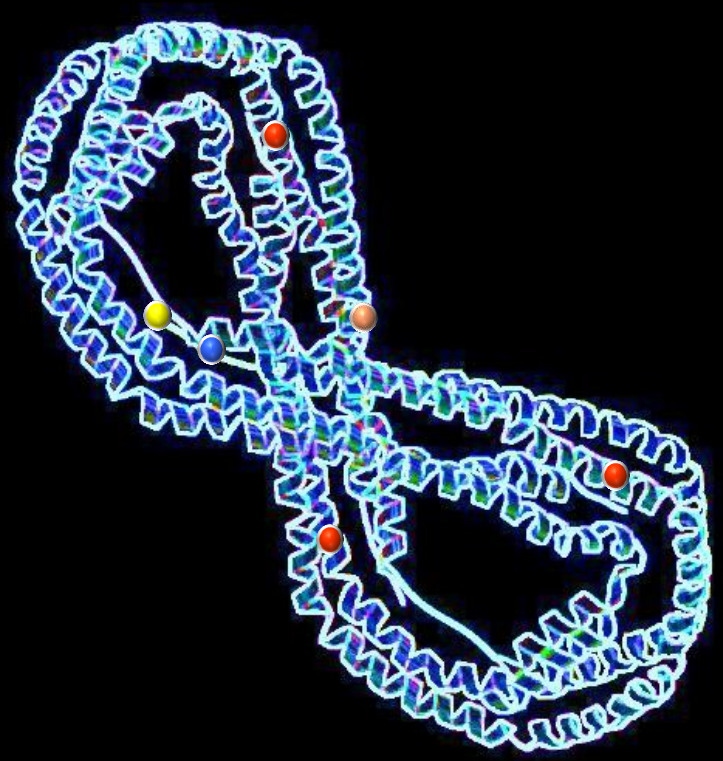
SEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTK*K*LNT*Q*

- ***Full-length (Direct)***
  - Ability to find & monitor additional forms



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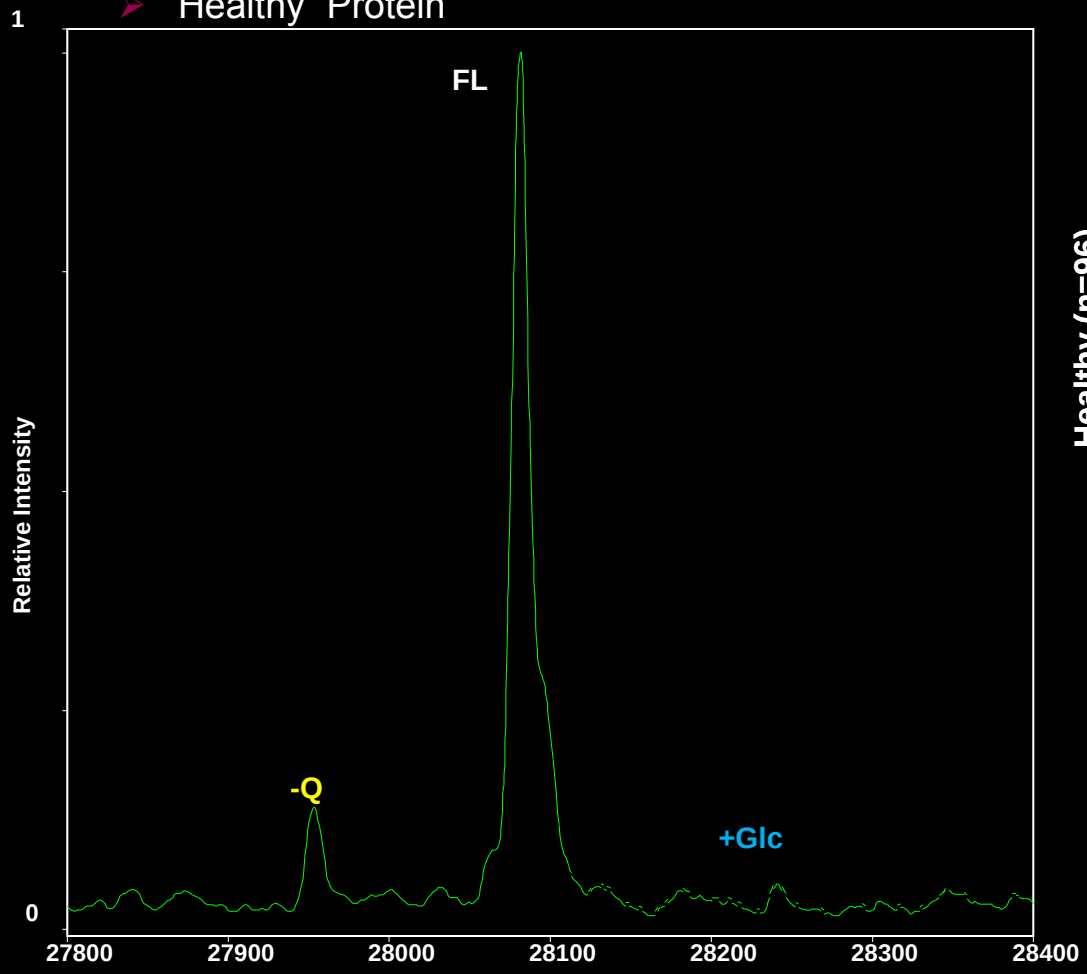
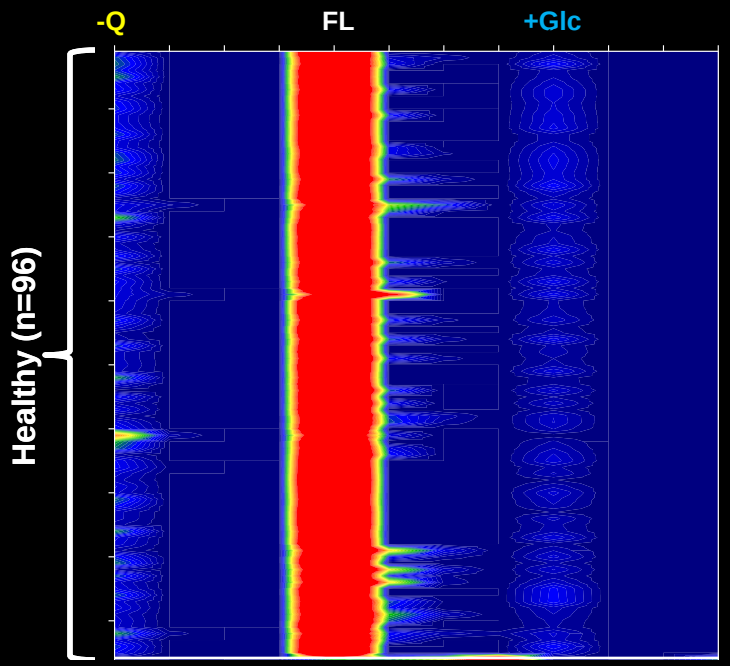
SEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTK*K*LNT*Q*

- ***Full-length (Direct)***
  - "Healthy" Protein



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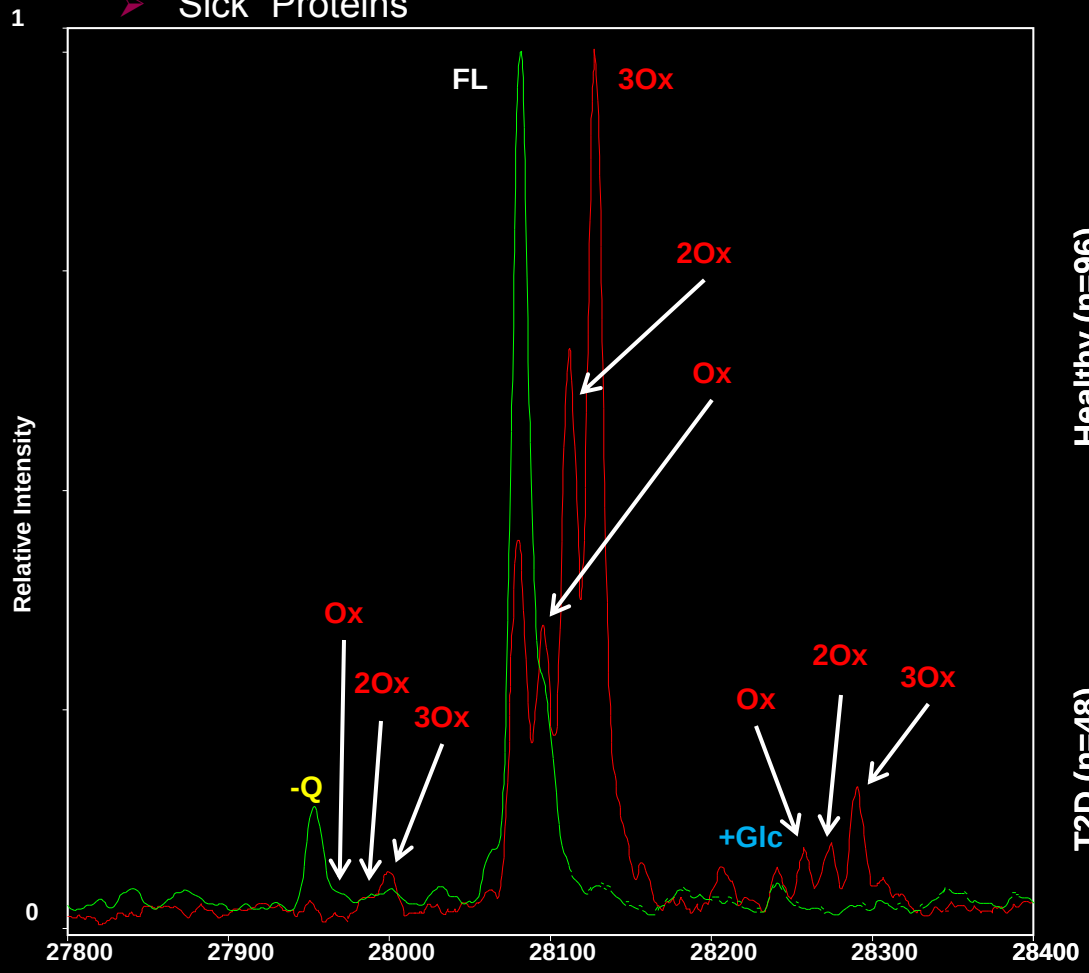
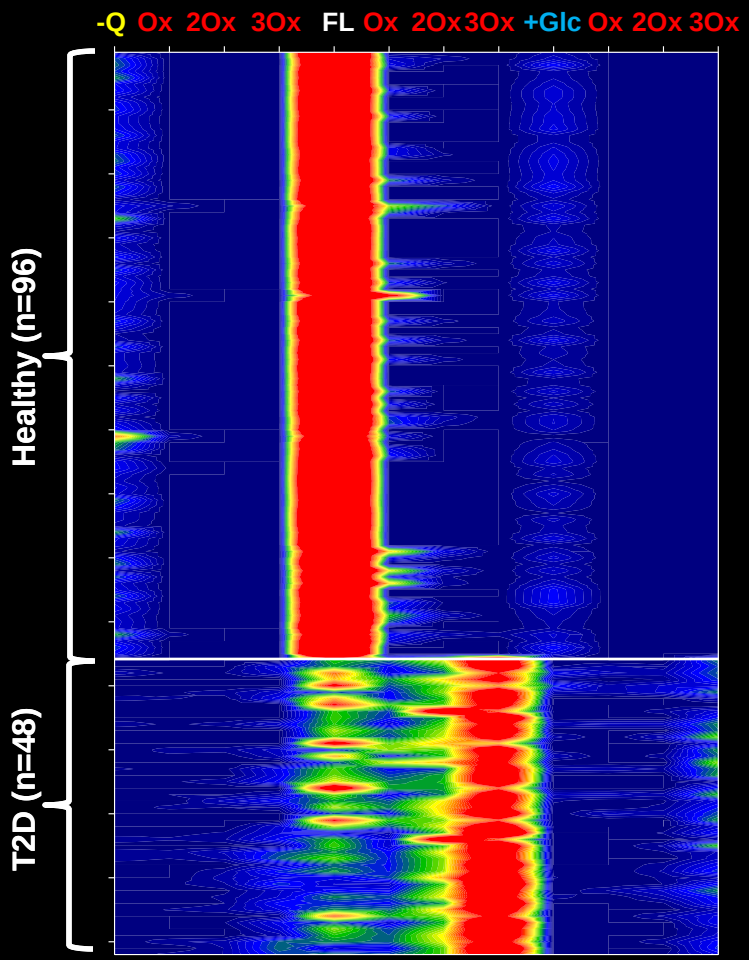
DFQKKWQEE*M*ELYRQKVEPLRAELQEGARQKLHELQEKLSPLGEE*M*RDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATEHLSTL
SEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTK*K*LNT*Q*

- ***Full-length (Direct)***
  - "Sick" Proteins



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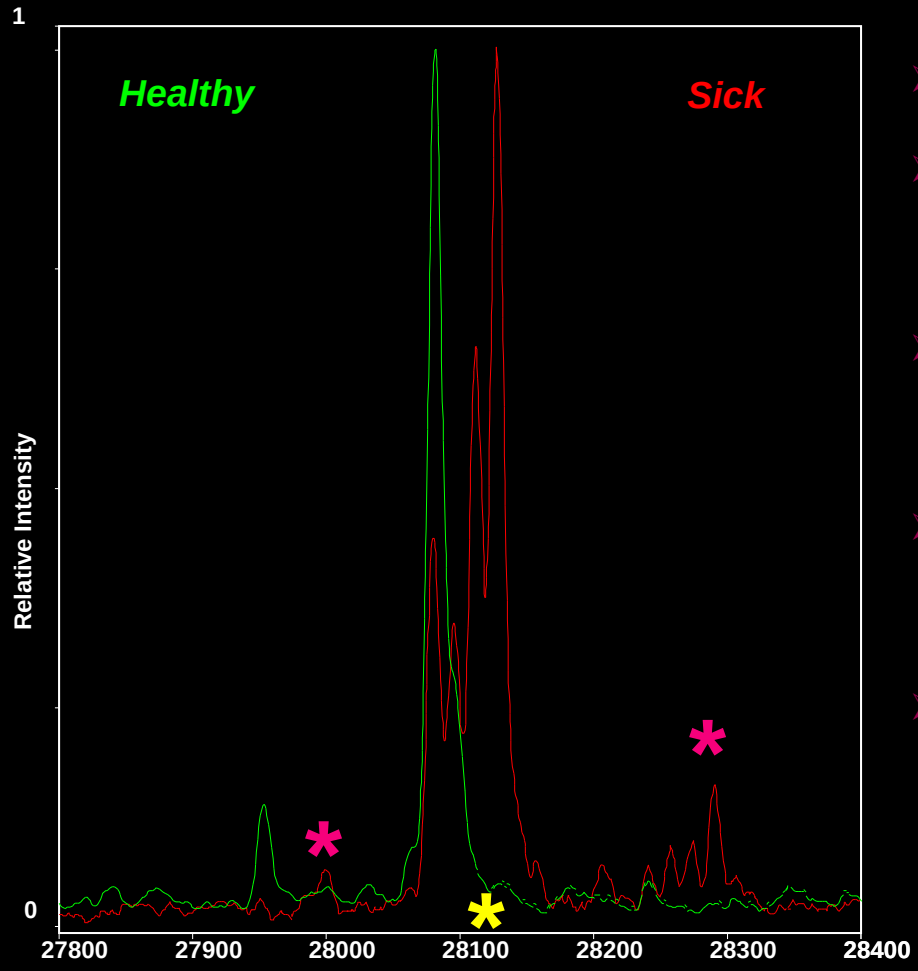
SEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTK*K*LNT*Q*



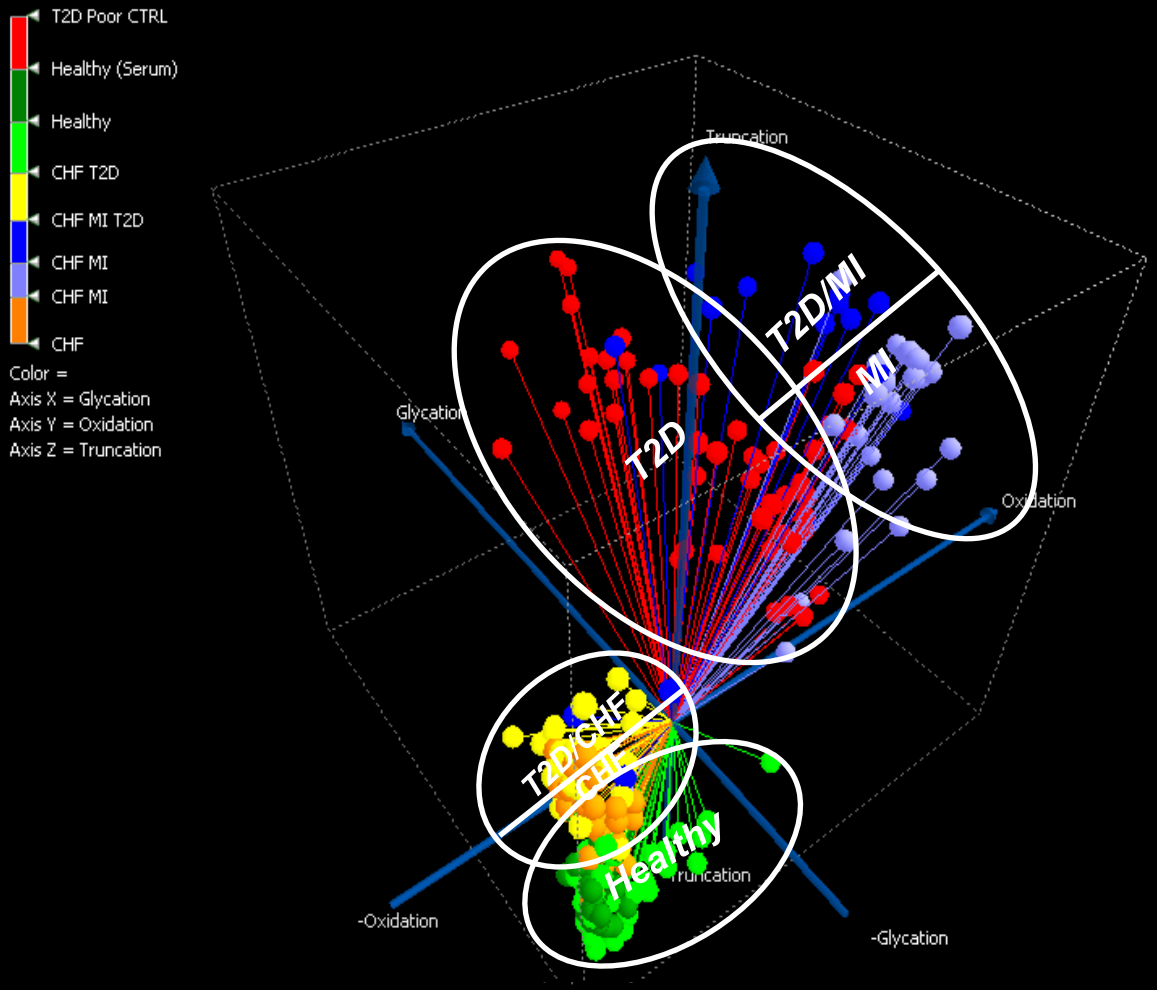

- ***"Sick Proteins"***
- "Multiplexed" Apo A1 Assay
  - Posttranslational Modifications
  - 12 Evident & Resolvable Forms
- Stoichiometry is Important
  - Ratio of Individual PTM to Total
  - Present LOD ~ 1%
- Data Captured by Surrogate Assays
  - Difficult to design to capture multiple PTM's
  - * Four PTM's contained in ~ 150 AA
- Anticipate Additional Low Level Variants
  - * Apo A1 with Five PTM's
  - Estimated at:
    - ~ 250 ppm (~ 5 ug/mL) in T2D
    - << in Healthy

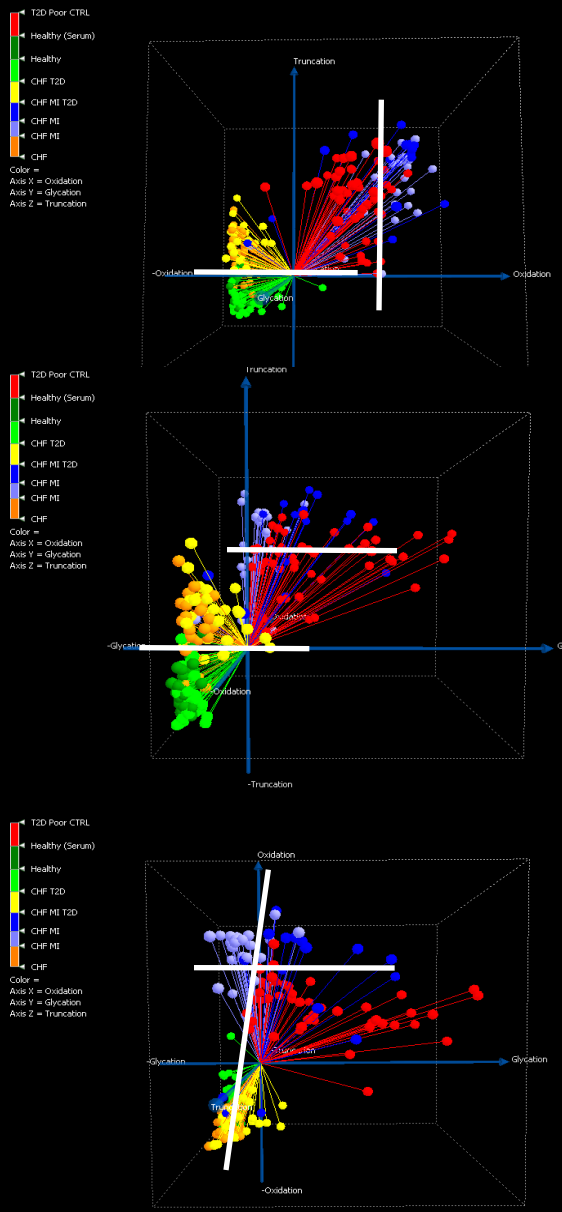
- **Expanded Cohort (> 200 Patients)**
  - **Healthy; T2D; T2D/MI; T2D/CHF; MI; CHF**
  - **Scounting Cohorts 20 – 50 Individuals/sub-type**
  - **13 Targets (> 2700 Assays Performed in Total)**
  - **139 Molecular Variants found in Population (~30,000 Data Points)**
  - **Principal Component Analysis & Biologically Supervised**

| Target | Conc (g/L) | Variants* | Category |
|---|---|---|---|
| Alb | 40 | 7 | FL, cys, glc, cysgly |
| Apo A1 | 2 | 12 | FL, glc, (3)ox, trunc |
| Apo C1 | $5x10^{-2}$ | 4 | FL, trunc, ox |
| b2m | $5x10^{-3}$ | 4 | FL, ox, glc, dk58 |
| C-peptide | $9x10^{-7}$ | 6 | FL, PM, (2)trunc |
| CysC | $1x10^{-3}$ | 7 | FL, OH-Pro, (2)trunc, (3)glc |
| GcG | $2x10^{-1}$ | 40 | 4 Allelic, glc, (3)glycos |
| Hemoglobin | - | 8 | (2)FL, HbS, glc |
| Insulin | $5x10^{-8}$ | 6 | FL, trunc, Novolog, (3)Lantus |
| RANTES | $3x10^{-6}$ | 21 | FL, trunc, glyc, glycos, ox |
| RBP | $1.5x10^{-2}$ | 4 | FL, trunc |
| SAP | $4x10^{-2}$ | 4 | FL, gylcos, trunc |
| TTR | $2x10^{-1}$ | 16 | FL, (3)PM, cys, cysgly, sulf |

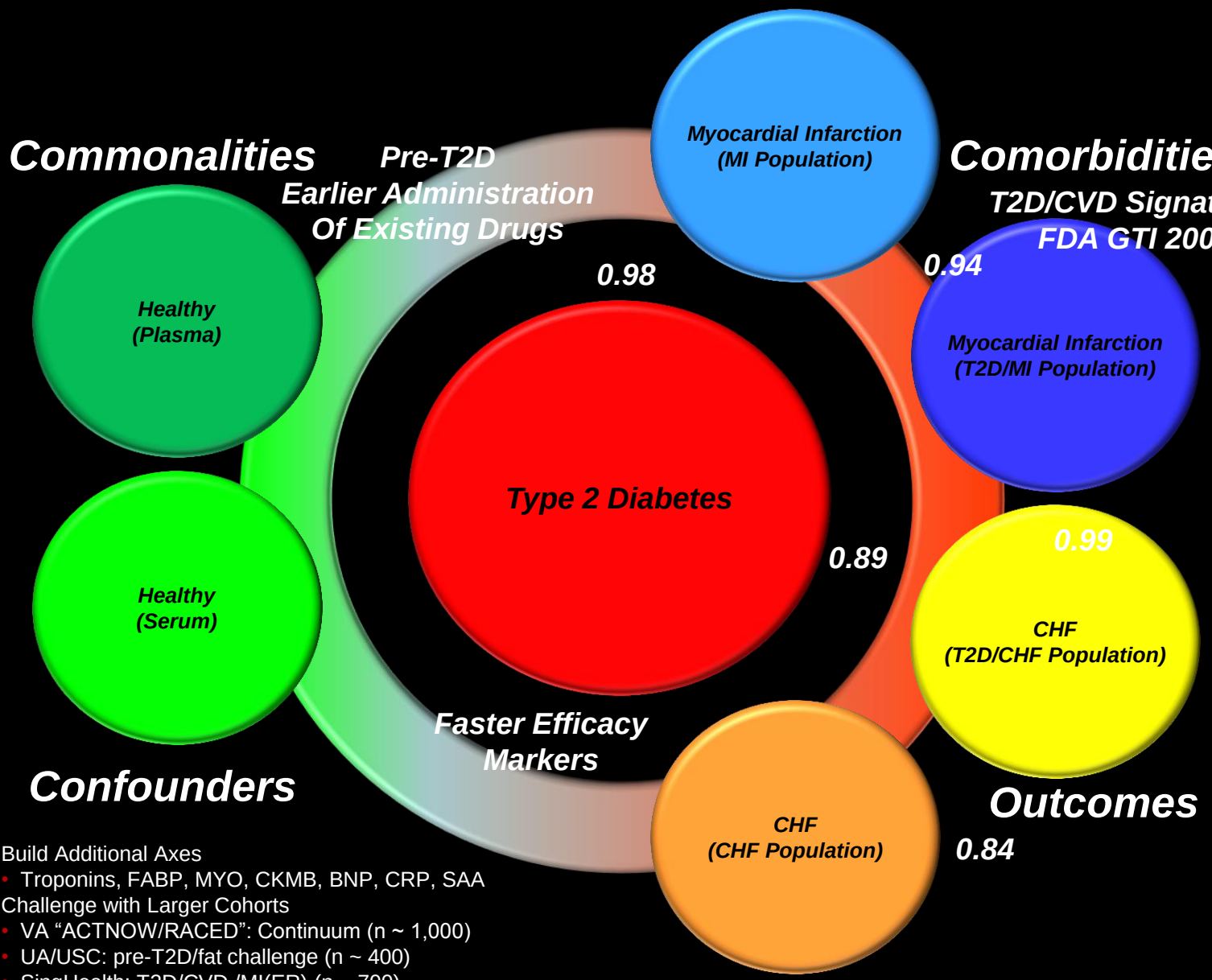
# *3-Dimensional View of T2DM/CVD: Biologically Supervised*

- Mixed Cohorts of Healthy – to – T2DM – to – CVD Outcome
  - 213 Individuals (Healthy, T2DM, CHF, MI)
  - 7 Assays per Individual (~ 1400 Assays)
  - 21 Determinates with Biological Supervision



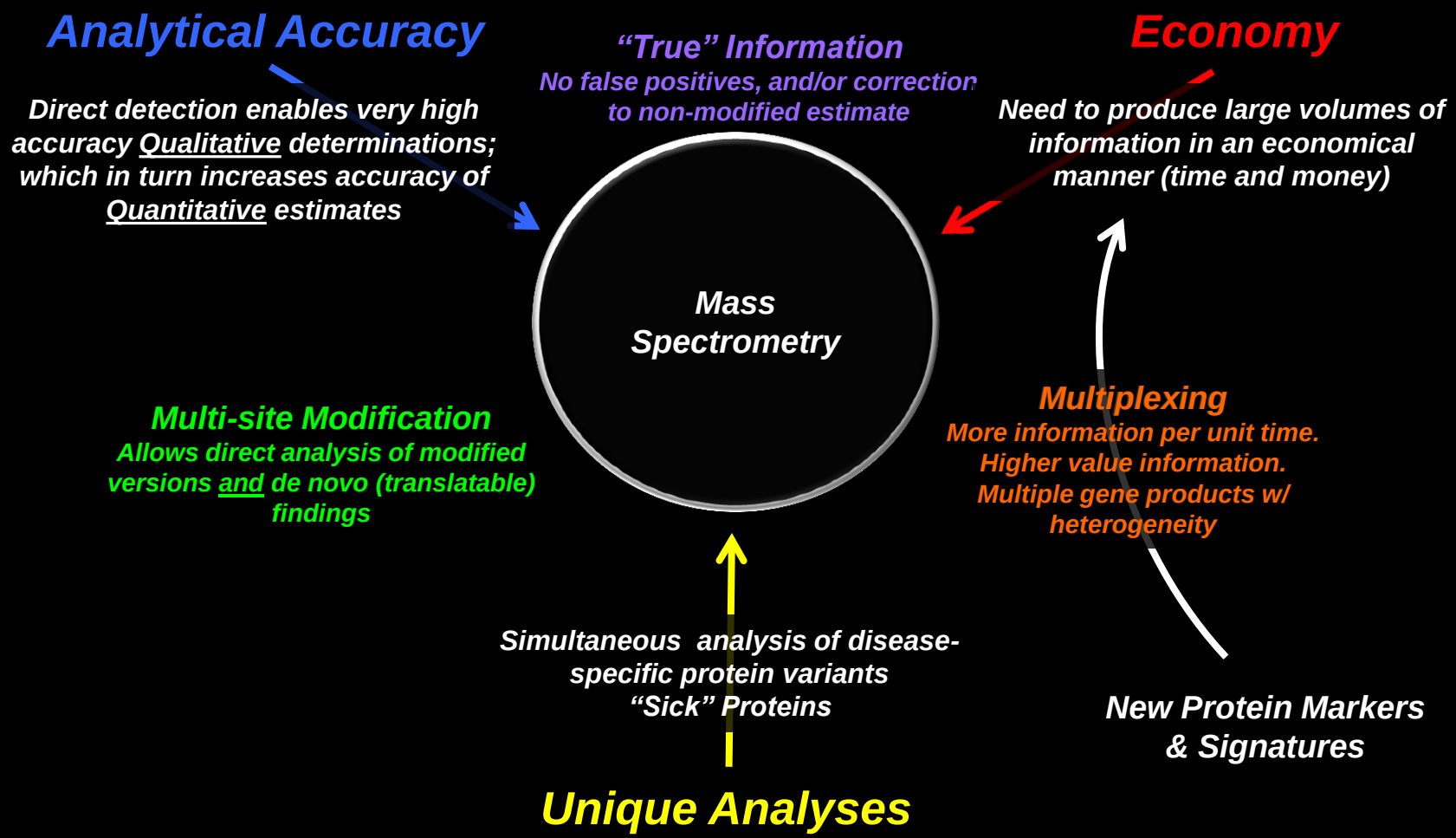



*Investigate the Healthy – T2D – CVD Continuum*

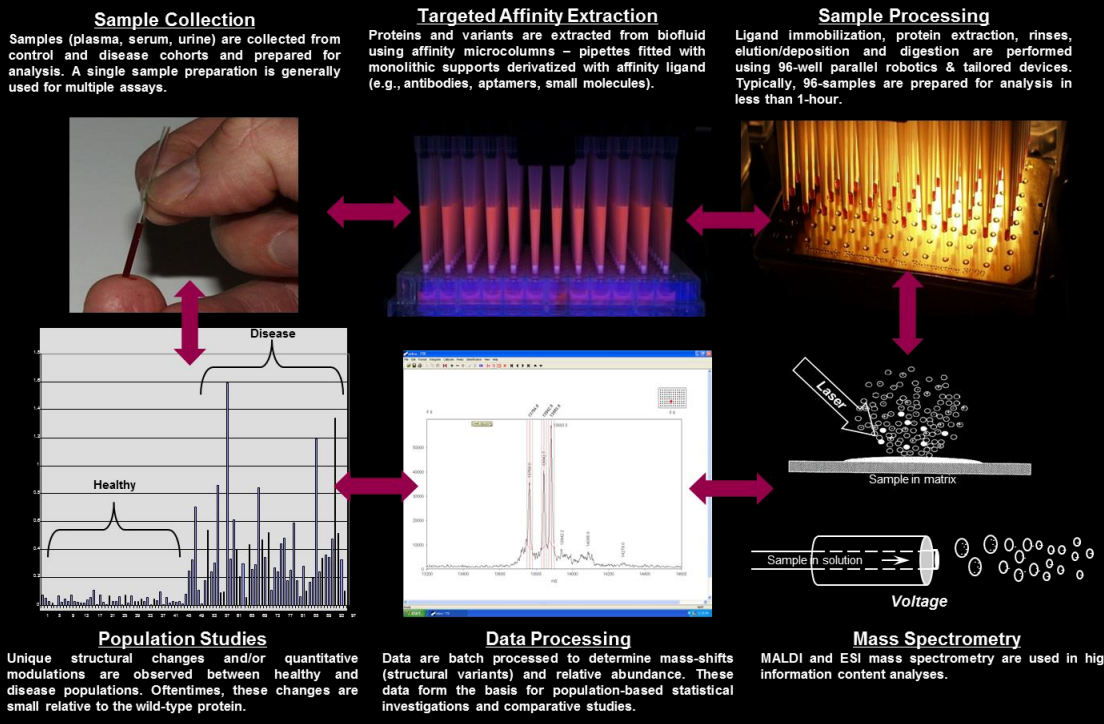


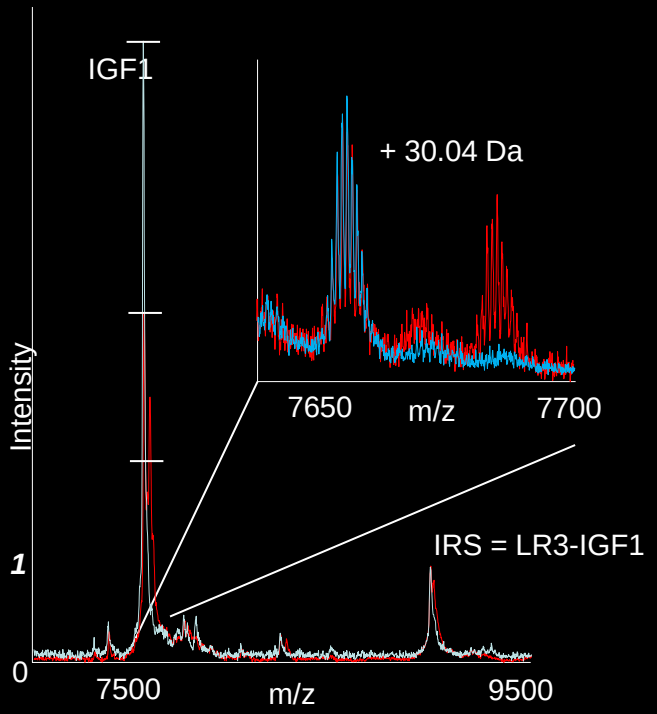

- Build Additional Axes
  - Troponins, FABP, MYO, CKMB, BNP, CRP, SAA
- Challenge with Larger Cohorts
  - VA “ACTNOW/RACED”: Continuum (n ~ 1,000)
  - UA/USC: pre-T2D/fat challenge (n ~ 400)
  - SingHealth: T2D/CVD /MI(ER) (n ~ 700)
  - Pfizer “ASCOT”: Longitudinal converters (n ~ 400)

# Randy's Unified Thoughts on Mass Spectrometry-Based Diagnostics



- ***IGF1 (1,056 Samples (T2D/CVD) + 96 Calibration Samples) – 24 hours***
  - *Diagnosing growth disorders, adult growth hormone deficiency & acromegaly/gigantism*
  - *Monitoring of recombinant human growth hormone treatment: FDA-approved treatment of IGFD (Increlex)*
  - *CPT Code 84305 – $64.24 Gross*




- ***IGF1 MSIA***
  - *Standard Error of Working Curves = 13.078 % (n=12; inter-curve (manual processing))*
  - *Dynamic Range > 300 (667 pM – 210 nM) (observed in samples 3.46 – 92.5 nM)*
  - *LOD < 500 pM; LOQ = 667 pM (40 uL plasma; Sample re-use for more assays, e.g., IGF2)*
- ***Quantification of wt-IGF-1 and Variant Detection/Correction***
  - *Point Mutations observed in ~ 1% of samples*
  - *e.g., Ala-Thr (dm = 30.03 Da)*

# *Next-Generation Clinical Mass Spectrometry Platform (CMSP)*

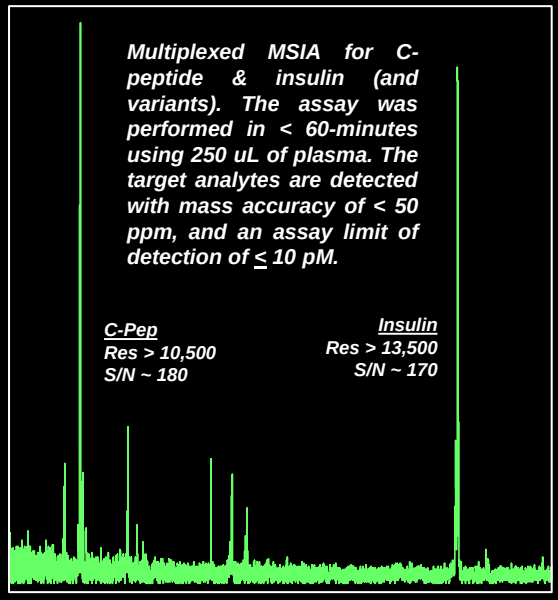
| *Current Technical Specifications For CMSP (from SimulTof Systems prototypes)* | | |
|---|---|---|
| **Category** | **Metric** | **Notes** |
| Throughput | Up to 3,000 analyses/day | Sample handling limits |
| Sample Consumption | 0.1 – 2 mL biofluid | Total sample for multiple assays |
| Cost (platform) | $100,000 - 300,000 | Cost on par with current non-MS platforms |
| Cost (assay) | < $15/data point | Cost of assay materials multiplexed preferred |
| User Interface | Graphic | Software paired w/assays |
| Ease of Use | Minimum User | Alpha prototype |
| Data Output | Numeric and graphic | Software paired w/assays |
| Size & Utilities | Benchtop (< 3 x 3 x 4 ft. d/w/h) 99/384/1584-well format/ 120V | Unobtrusive, operating on common utilities |
| Data Acquisition | Automated | < 10-seconds/sample (10-50k laser shots) |
| Cost (platform) | $150,000 - 200,000 | Depends on final specifications |
| Resolution/Accuracy /Range | > 20,000/ ~ 5 ppm/ 500 – 150,000 Da | Exceeds research-grade instruments |
| Limit of Detection | Low pM Sub-femtomole | Exceeds research-grade instruments |

| *Preliminary Candidates for Assay Development* | | |
|---|---|---|
| **Target*** | **Uses/Known Variants**** | **Price ($)***** |
| GIP | B-cell stimulation / truncated forms (e.g., GIP(3-42)) | 280.00 |
| Glucagon | Glucagonomas, hyper- or hypoglycemia / truncated forms | 175.00 |
| C-peptide | Hypoglycemia, insulinoma, islet cell transplant / C-pep(3-31 | 125.00 |
| Insulin | Insulinoma/ exogenous forms (e.g., insulin glargine, aspart) | 112.00 |
| Angiotensin I | Renin activity / AngII precursor | 175.00 |
| Angiotensin II | Hypertension / vasoconstriction / AngIII precursor | 300.00 |
| IGF-1 | Growth disorders/ Point mutations | 172.00 |
| PTH | Hypercalcemia, renal failure / PTH-(1-84) vs PTH-(7-84) | 200.00 |
| FGF-23 | AD hypophosphatemic rickets / splice variants | 194.00 |
| ACTH | Hypocorticolism / truncated (e.g., ACTH(1-11), ACTH(1-34)) | 230.00 |

* Gastro Inhibitory Peptide (GIP); Insulin-like Growth Factor 1 (IGF-1), Parathyroid Hormone (PTH), Fibroblast Growth Factor 23 (FGF-23), Adrenocorticotropic Hormone (ACTH).

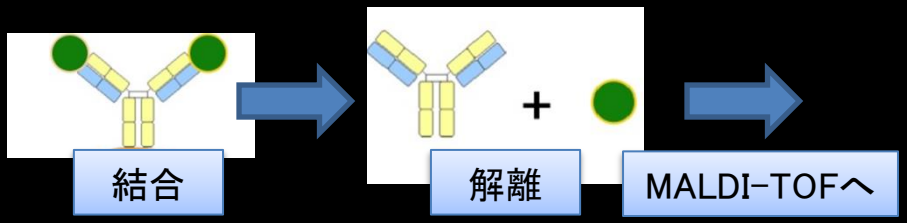
** From literature search, not meant to be exhaustive.

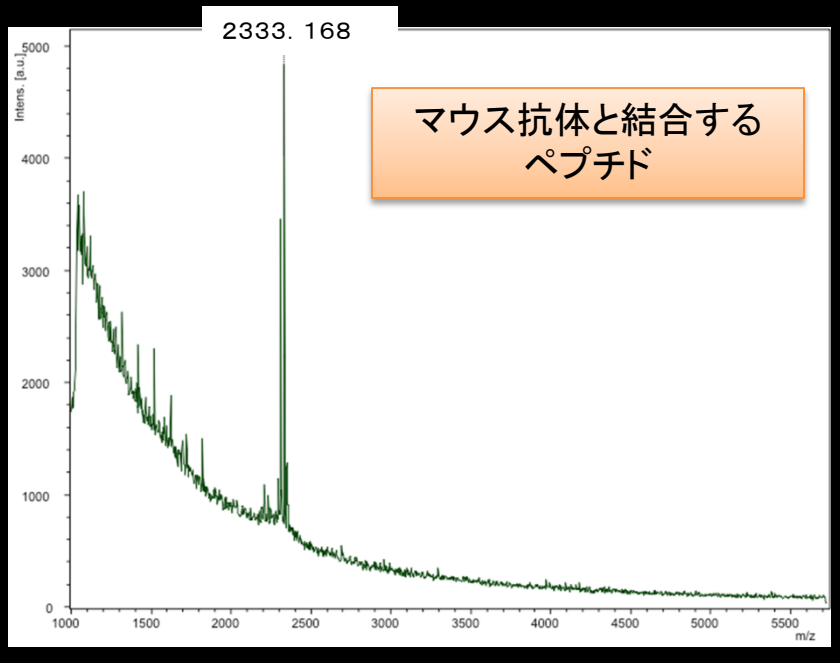
*** Fees charged for service (Mayo Medical Laboratories), unit cost are ~10-15% of these values.

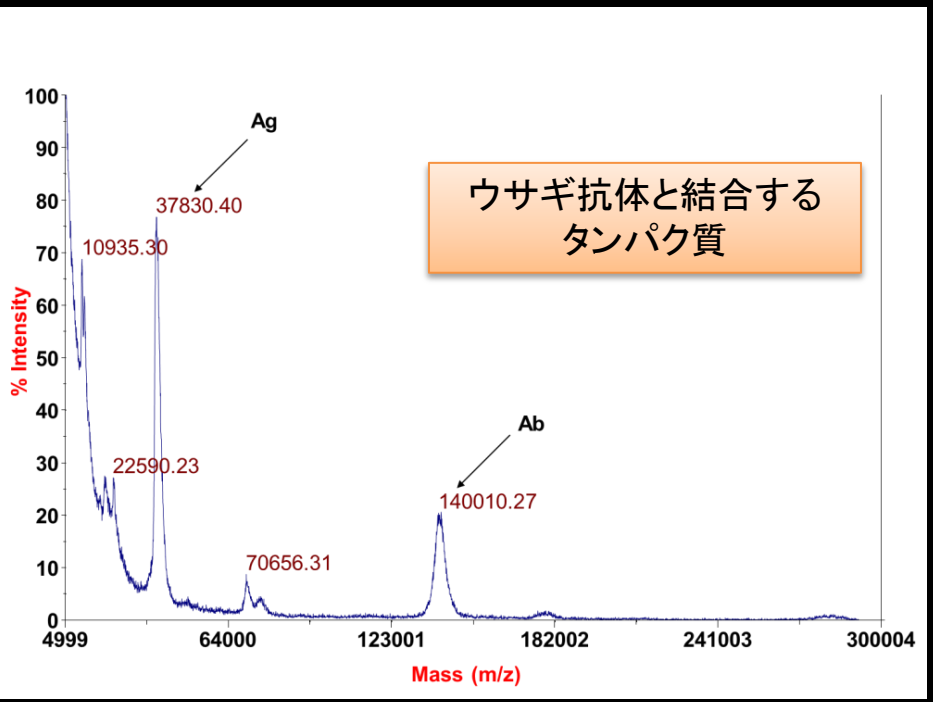


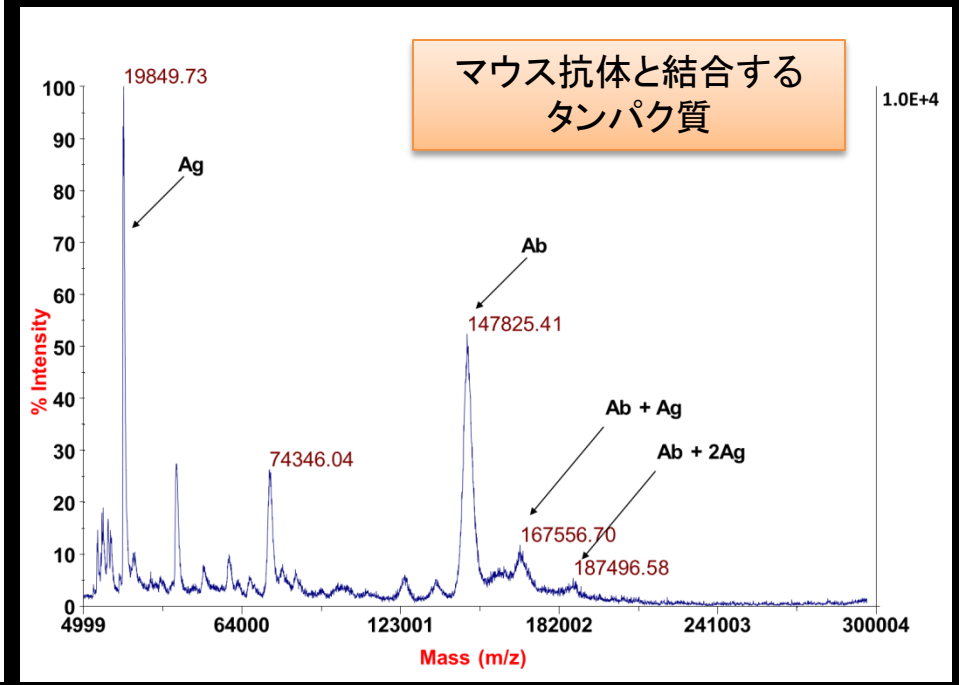
# SkiPro

細胞培養液をアナライトで、TOF-MSへ

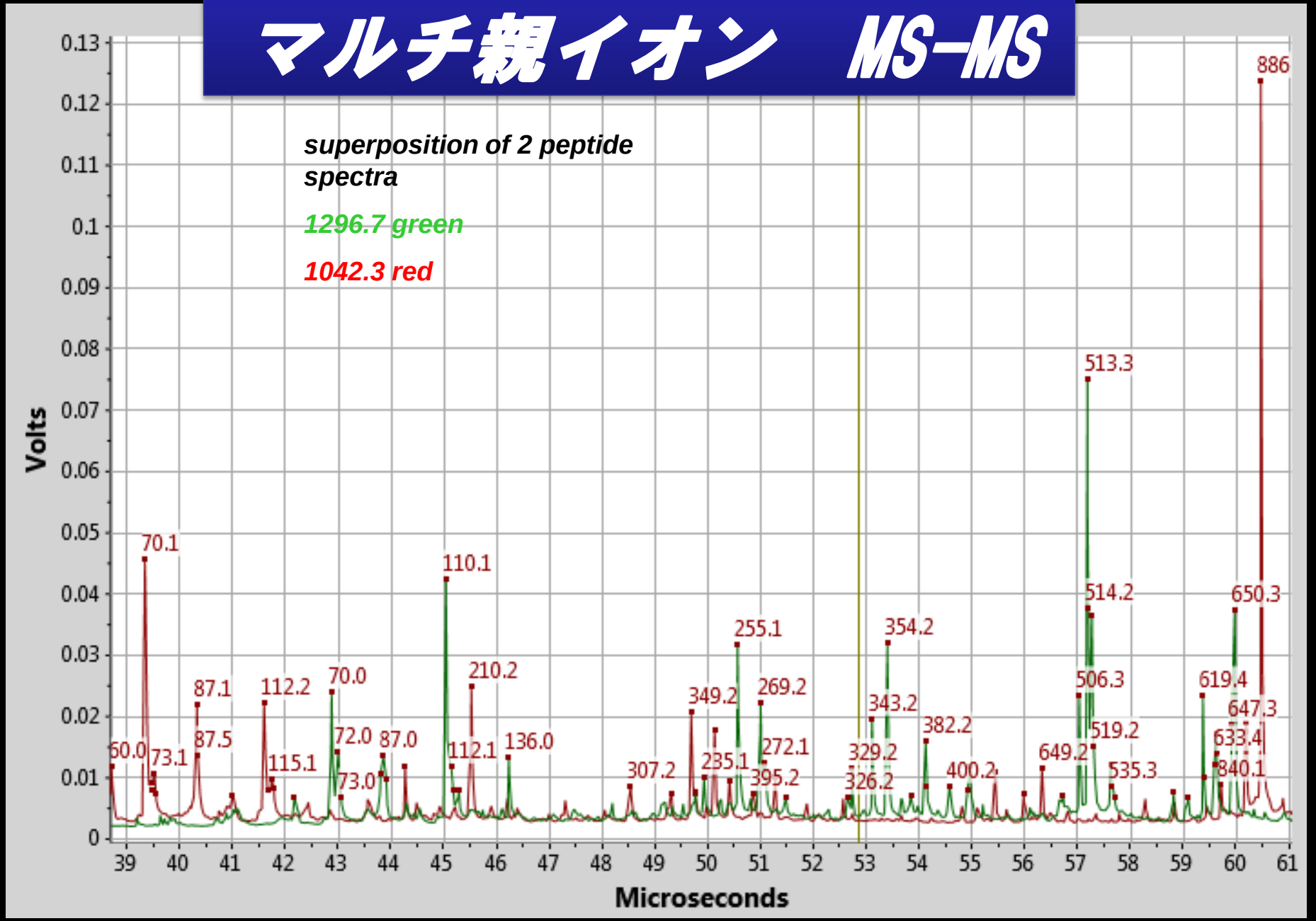


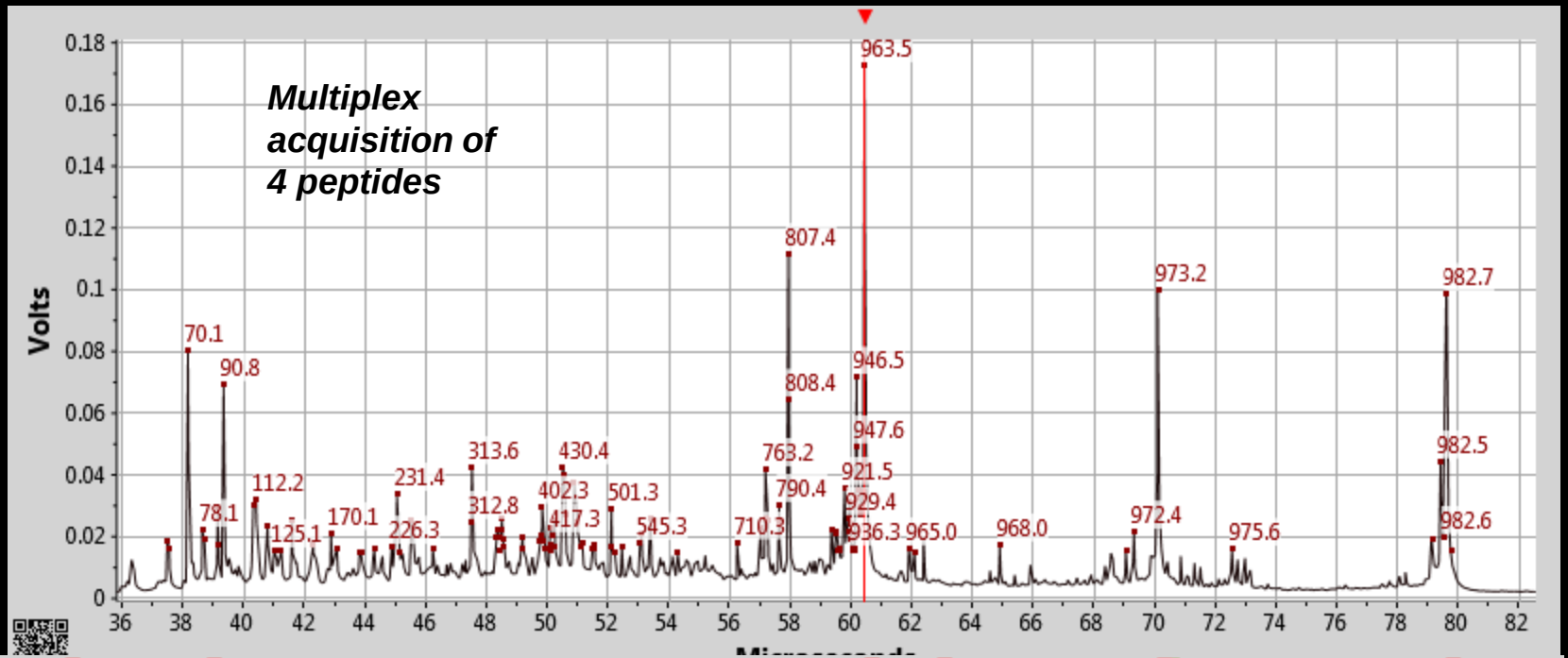



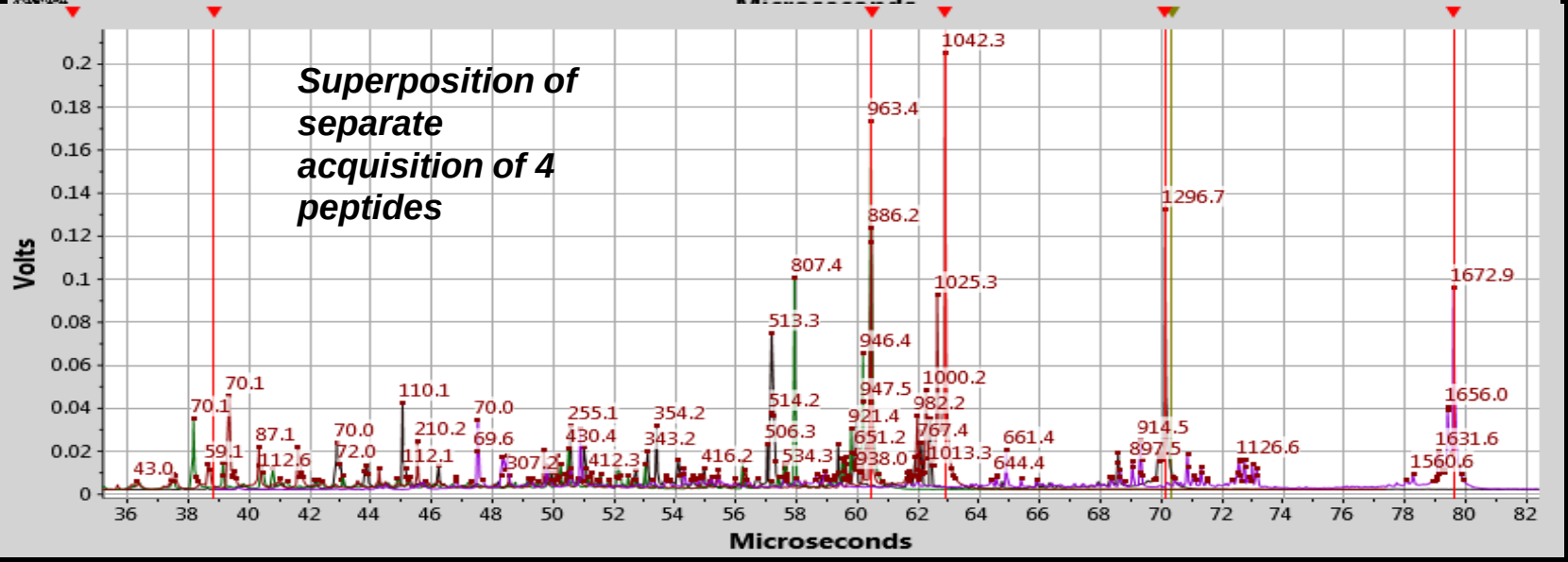



# マルチ親イオン　MS-MS




Multiplex acquisition of 4 peptides
Volts
963.5
807.4
973.2
982.7
70.1
90.8
946.5
808.4
947.6
313.6
430.4
763.2
982.5
231.4
921.5
112.2
402.3
501.3
790.4
929.4
78.1
312.8
417.3
545.3
710.3
936.3
965.0
968.0
972.4
975.6
982.6
125.1
170.1
226.3


Superposition of separate acquisition of 4 peptides
Volts
Microseconds
1042.3
963.4
1296.7
886.2
807.4
1025.3
1672.9
513.3
946.4
1000.2
70.1
947.5
982.2
1656.0
514.2
921.4
914.5
110.1
255.1
354.2
506.3
651.2
767.4
661.4
1631.6
87.1
210.2
430.4
343.2
534.3
938.0
1013.3
644.4
897.5
1126.6
1560.6
59.1
112.6
112.1
307.2
412.3
416.2
43.0
72.0
69.6