PROTEIN METRICS

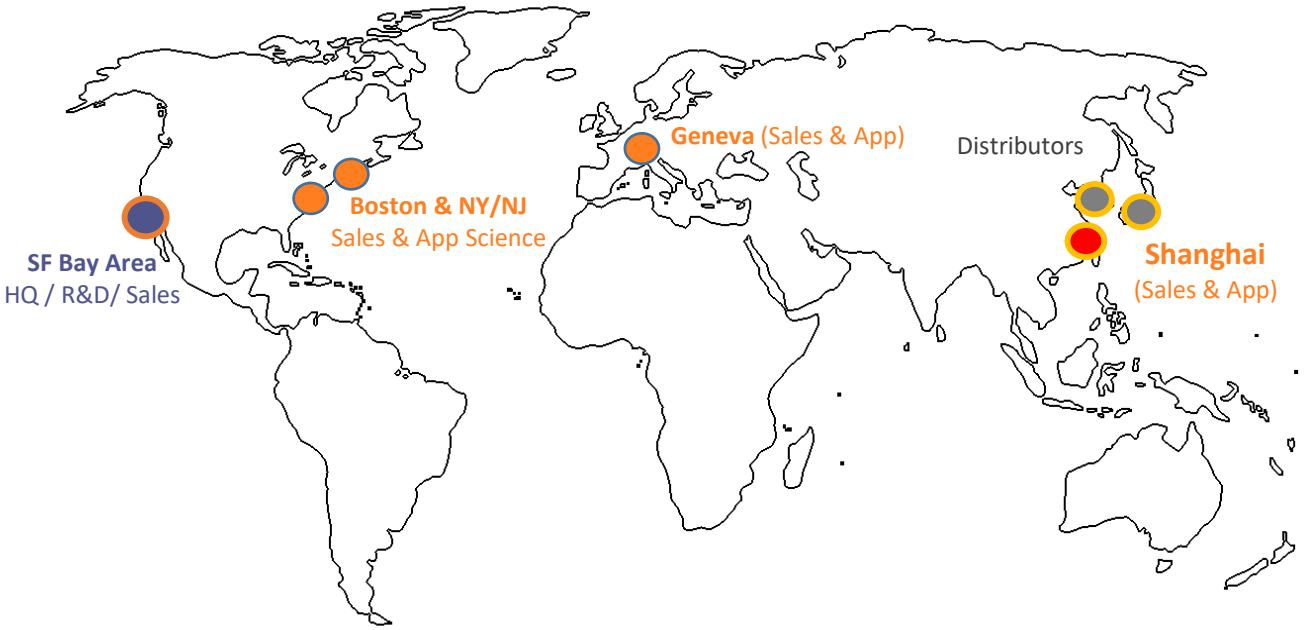
Software for Oligonucleotide Analysis by Mass Spectrometry

Marshall Bern

October 10th 2019

SUMS, Stanford CA

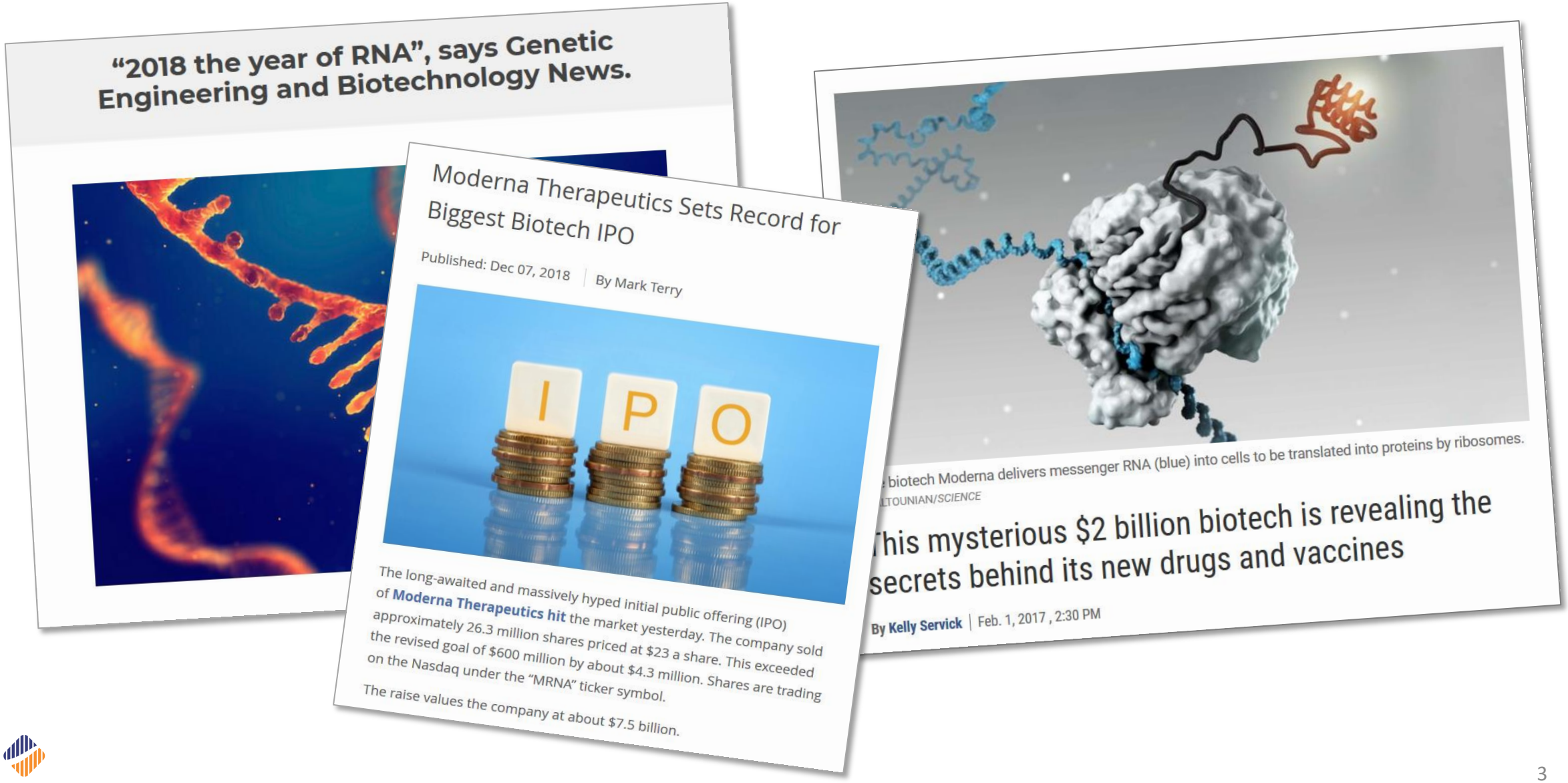
Protein Metrics Inc. - Overview



- Software company founded in 2011
  (Cupertino, same address as Apple circa 1978)
- In almost all major biopharma companies
- > 100 academic laboratories
- 9 SBIR / STTR grants from NIH NIGMS

PROTEIN METRICS

Synthetic oligonucleotides are **hot** !

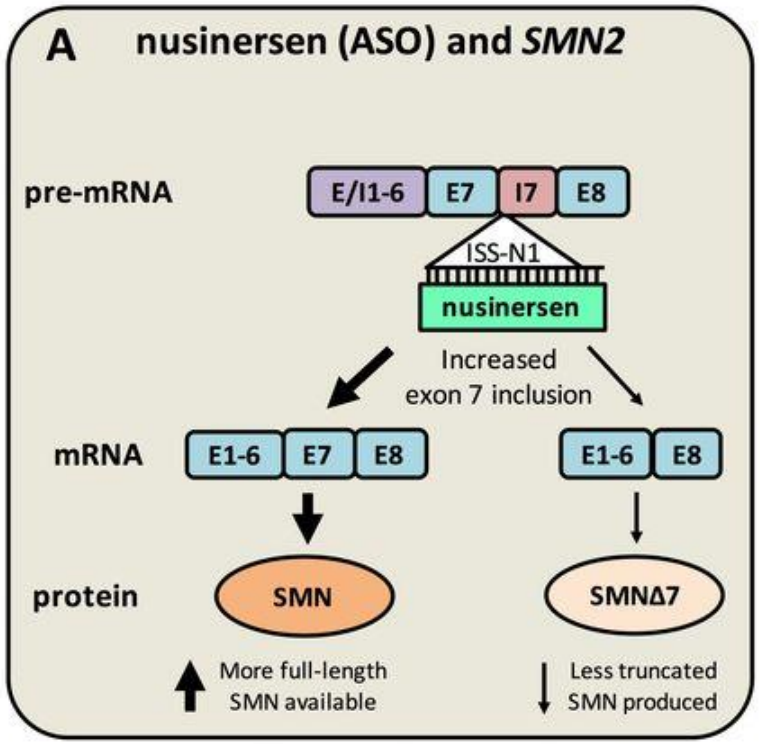


PROTEIN METRICS

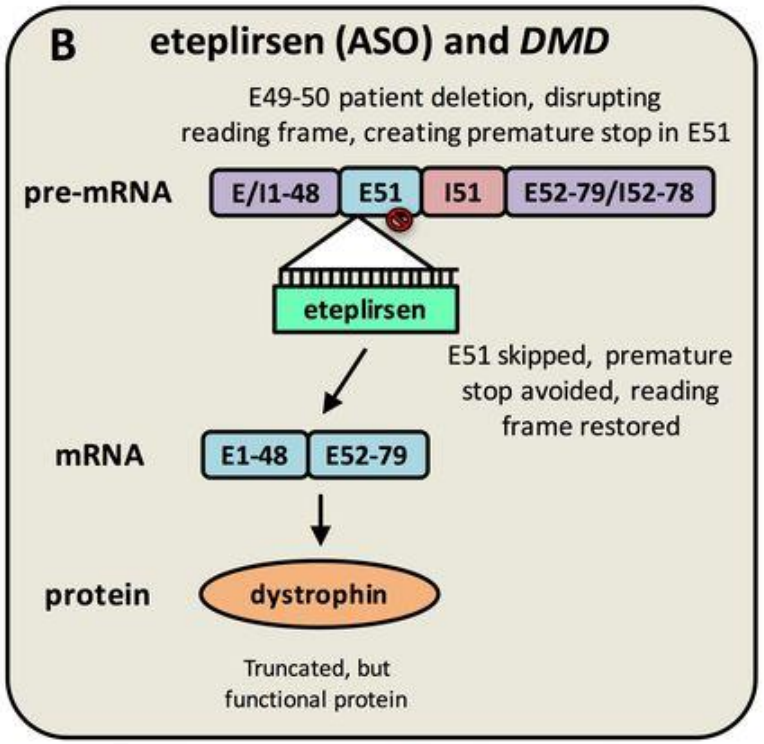
Drug Mechanisms

Single-stranded antisense oligos (ASO) can repair bad genes

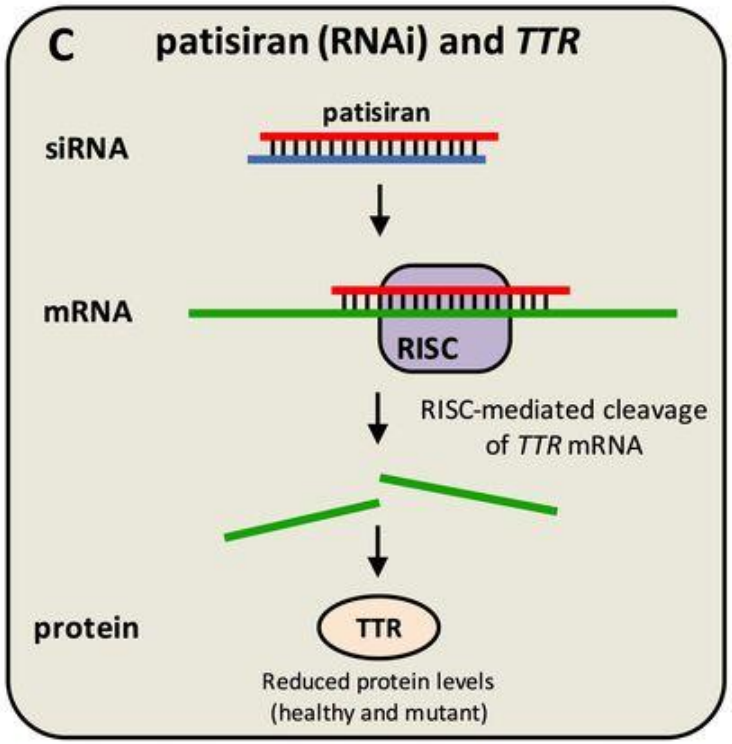
Double-stranded RNAi can kill unwanted mRNA's



spinal muscular atrophy

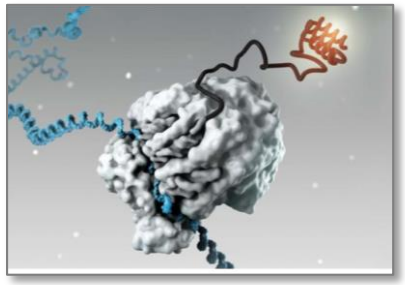


Duchenne muscular dystrophy



familial amyloid polyneuropathy

… and someday mRNAs will make new proteins!



PROTEIN METRICS

Eteplirsen

From Wikipedia, the free encyclopedia

Eteplirsen (brand name **Exondys 51**) is a medication to treat, but not cure, some types of Duchenne muscular dystrophy (DMD), caused by a specific mutation. Eteplirsen only targets specific mutations and is useful in about 1.5% of cases.[1][2] DMD ≈ 1 in 3500 male births

Eteplirsen was designed and developed by Sarepta Therapeutics. After a controversial debate surrounding the efficacy of the drug, during which two FDA review panel members resigned in protest, eteplirsen received accelerated approval from the US Food and Drug administration in late 2016.[3][4] A year's worth of treatment is expected to cost approximately $300,000.[5]

CTCCAACATCAAGGAAGATGGCATTTCTAG

NDA 206488 David B. Hawver, Ph.D.

Chemical Name

RNA, [*P*-deoxy-*P*-(dimethylamino)] (2′,3′-dideoxy-2′,3′-imino-2′,3′-seco) (2′a→ 5′) (C-m^5U-C-C-A-A-C-A-m^5U-C-A-A-G-G-A-A-G-A-m^5U-G-G-C-A-m^5U-m^5U-m^5U-C-m^5U-A-G), 5′-[*P*-[4-[[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]carbonyl]-1-piperazinyl]-*N*,*N*-dimethylphosphonamidate]

Molecular Formula

$C_{364}H_{569}N_{177}O_{122}P_{30}$

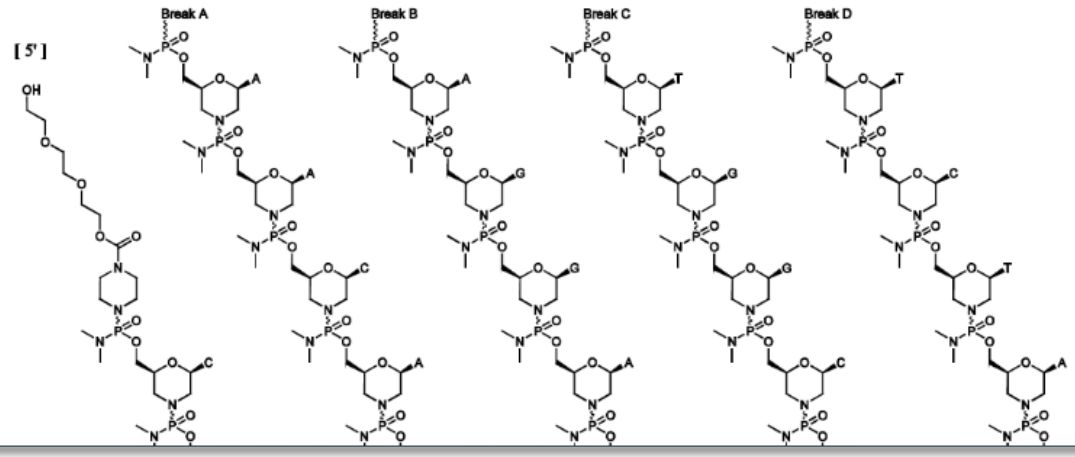
Molecular Weight

10305.7

What's this?

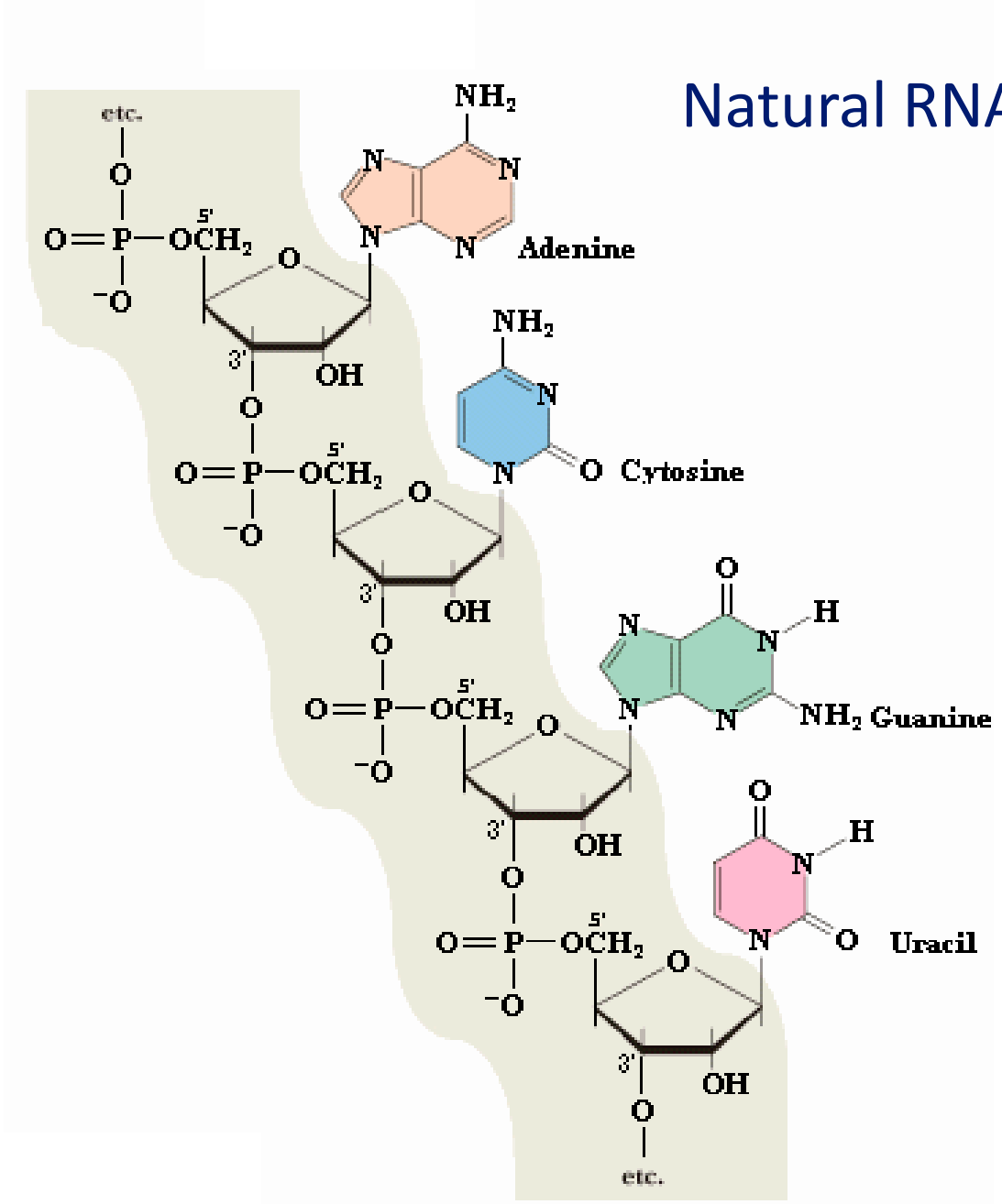
Biochemical Description and Structure

Eteplirsen is a charge-neutral phosphorodiamidate morpholine oligomer (PMO) consisting of a sequence of 30 of the following four nucleobases: 5-methyluracil (m^5U; thymine; T), adenine (A), cytosine (C), and guanine (G).

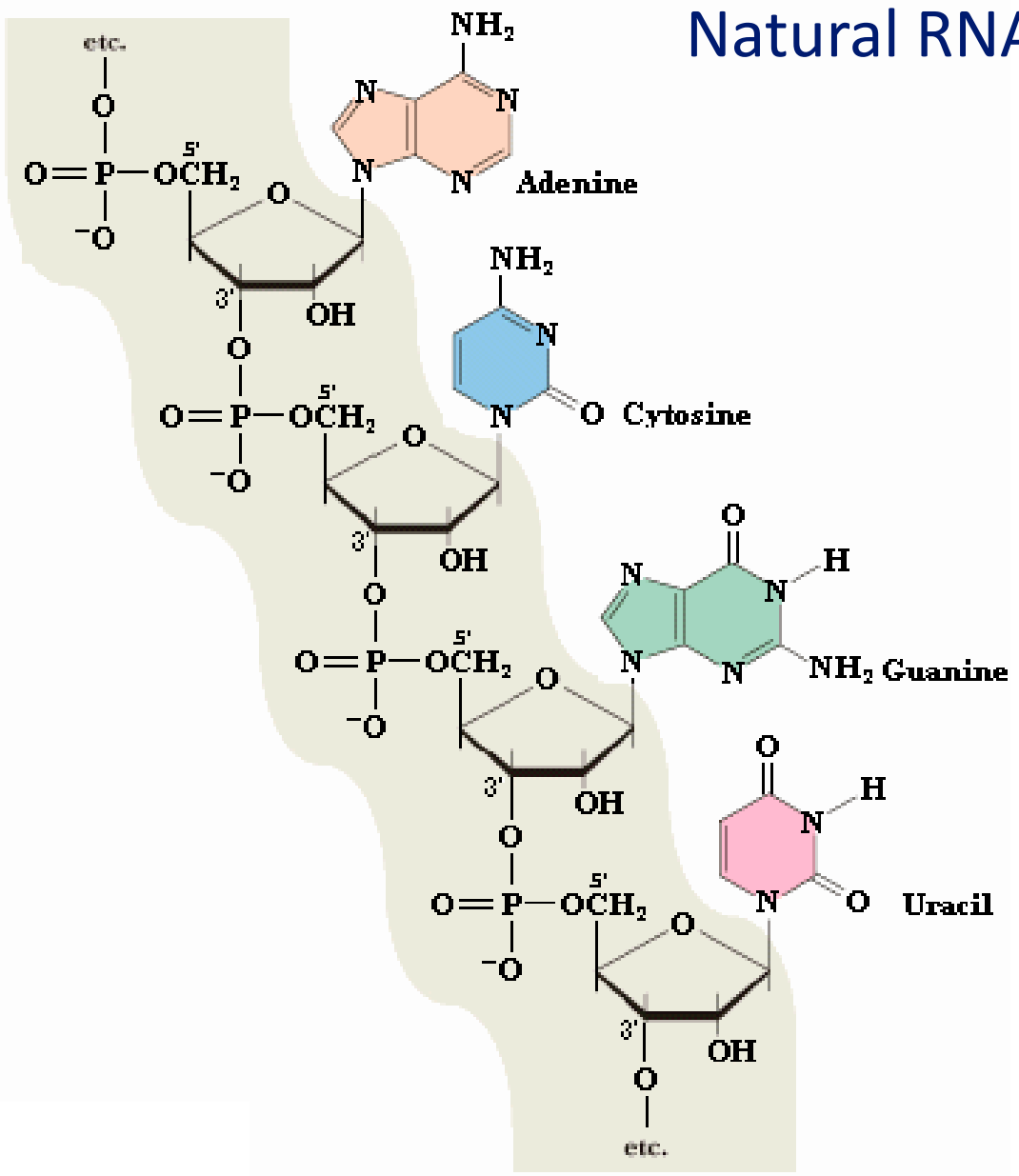


PROTEIN METRICS

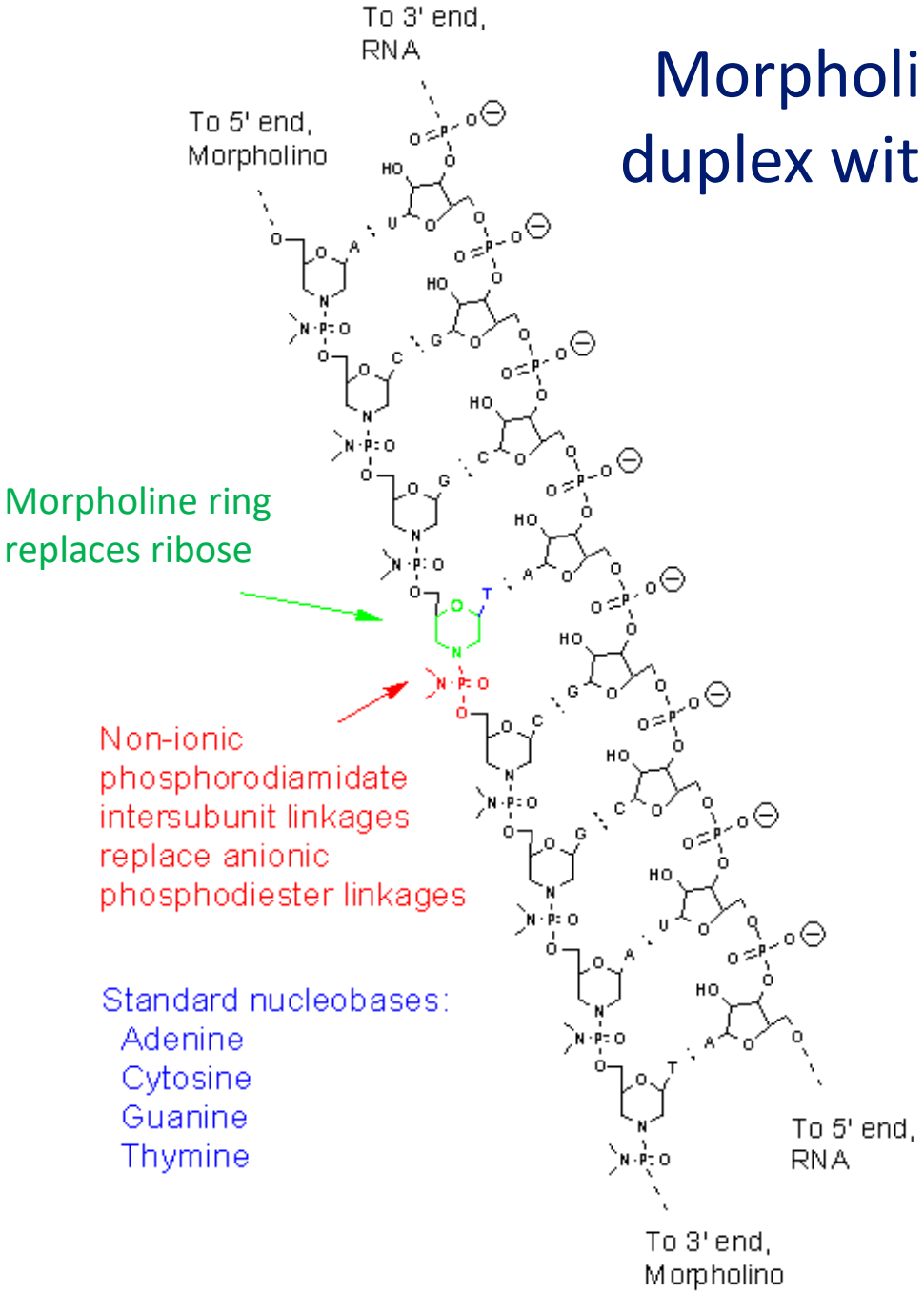
Natural RNA



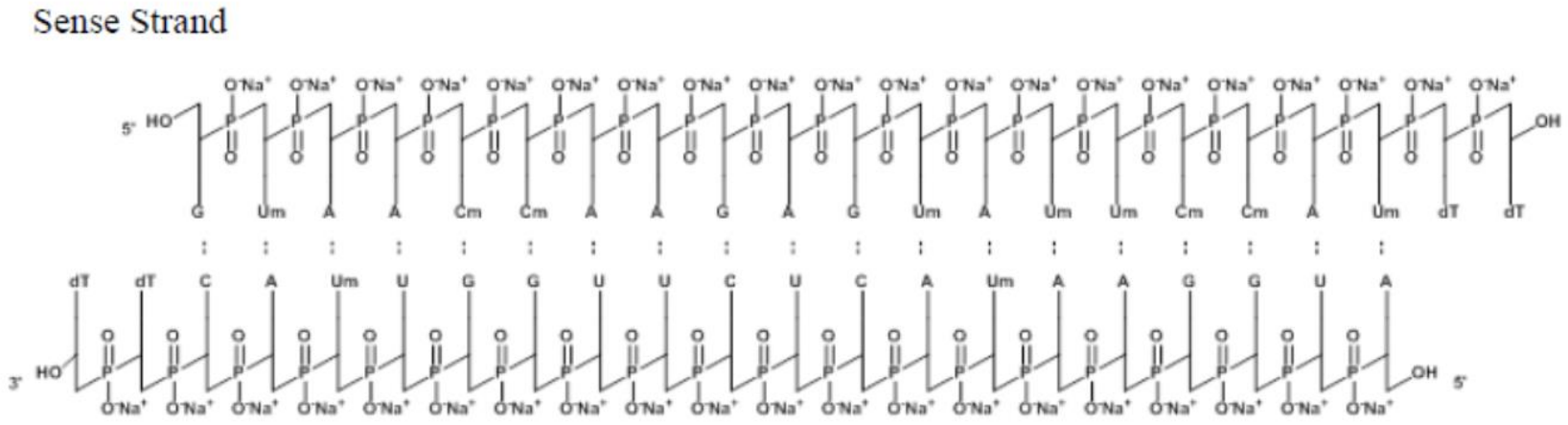
Natural RNA



Morpholino in duplex with RNA



Patisiran



Antisense Strand

Sense	5'	G	Um	A	A	Cm	Cm	A	A	G	A	G	Um	A	Um	Um	Cm	Cm	A	Um	dT	dT
Antisense	3'	dT	dT	C	A	Um	U	G	G	U	U	C	U	C	A	Um	A	A	G	G	U	A

The chemical structures and physical properties of patisiran and the single strand intermediates were elucidated and confirmed by a combination of LC-MS, MS-MS sequence confirmation, thermal dependent UV absorbance, SE-HPLC UV, FAAS, UV absorption, UV spectroscopy, ^{1}H-NMR, Imino-^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR, FTIR, circular dichroism, differential scanning calorimetry and thermogravimetric analysis.

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

26 July 2018
EMA/554262/2018
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

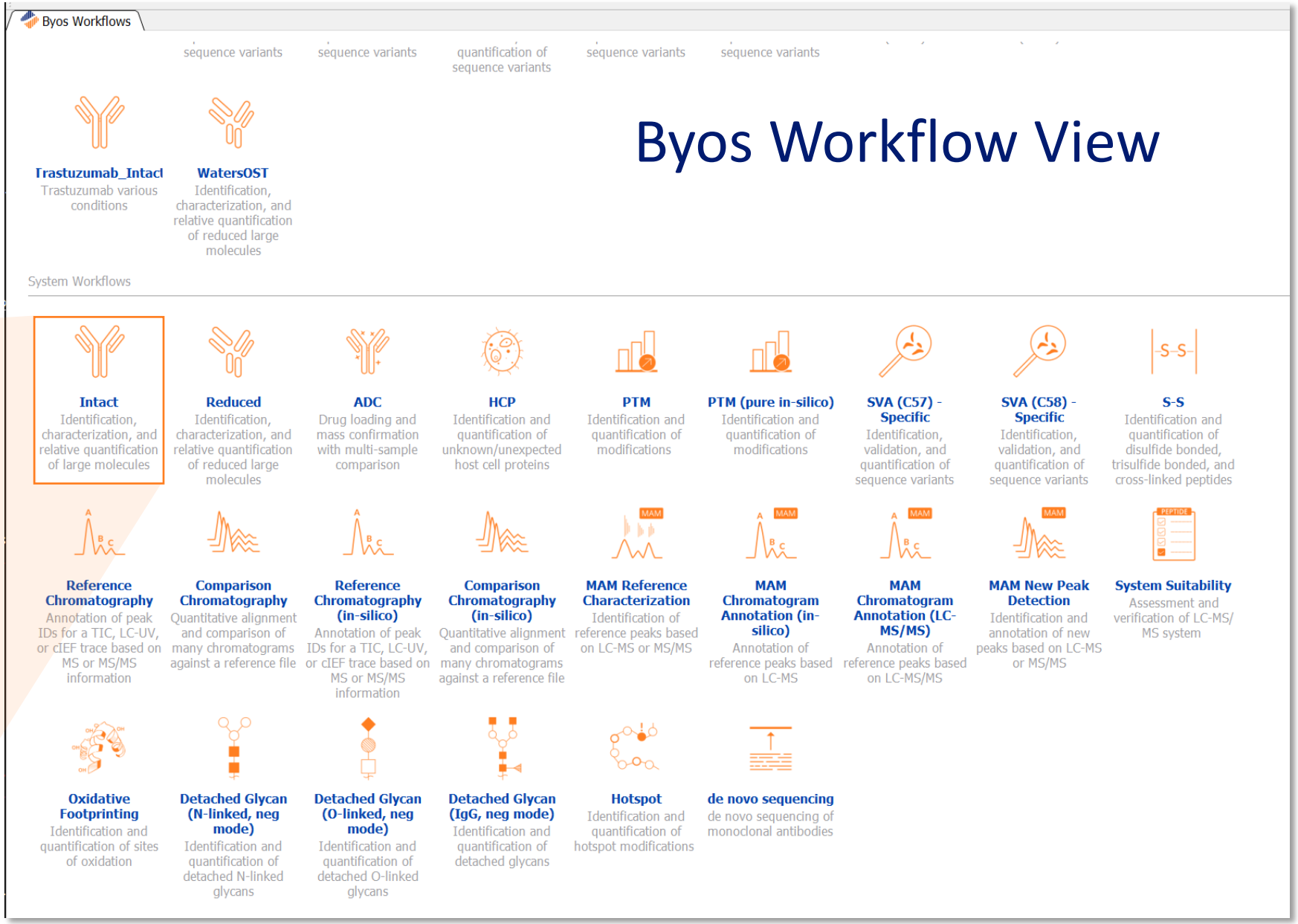
Onpattro

International non-proprietary name: patisiran

PROTEIN METRICS

LC-MS Analysis

Byos Workflow View

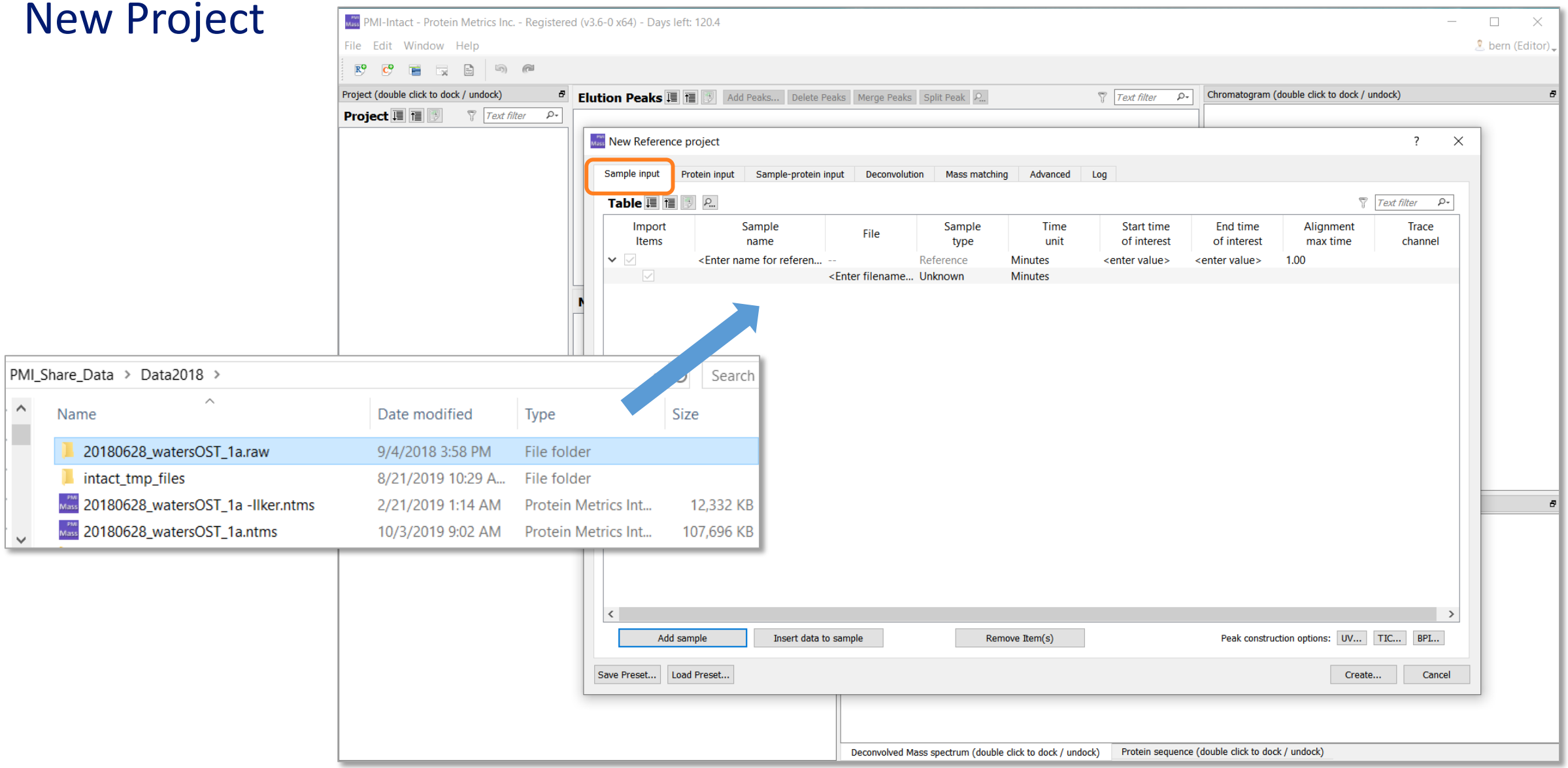


Intact

Identification, characterization, and relative quantification of large molecules

PROTEIN METRICS

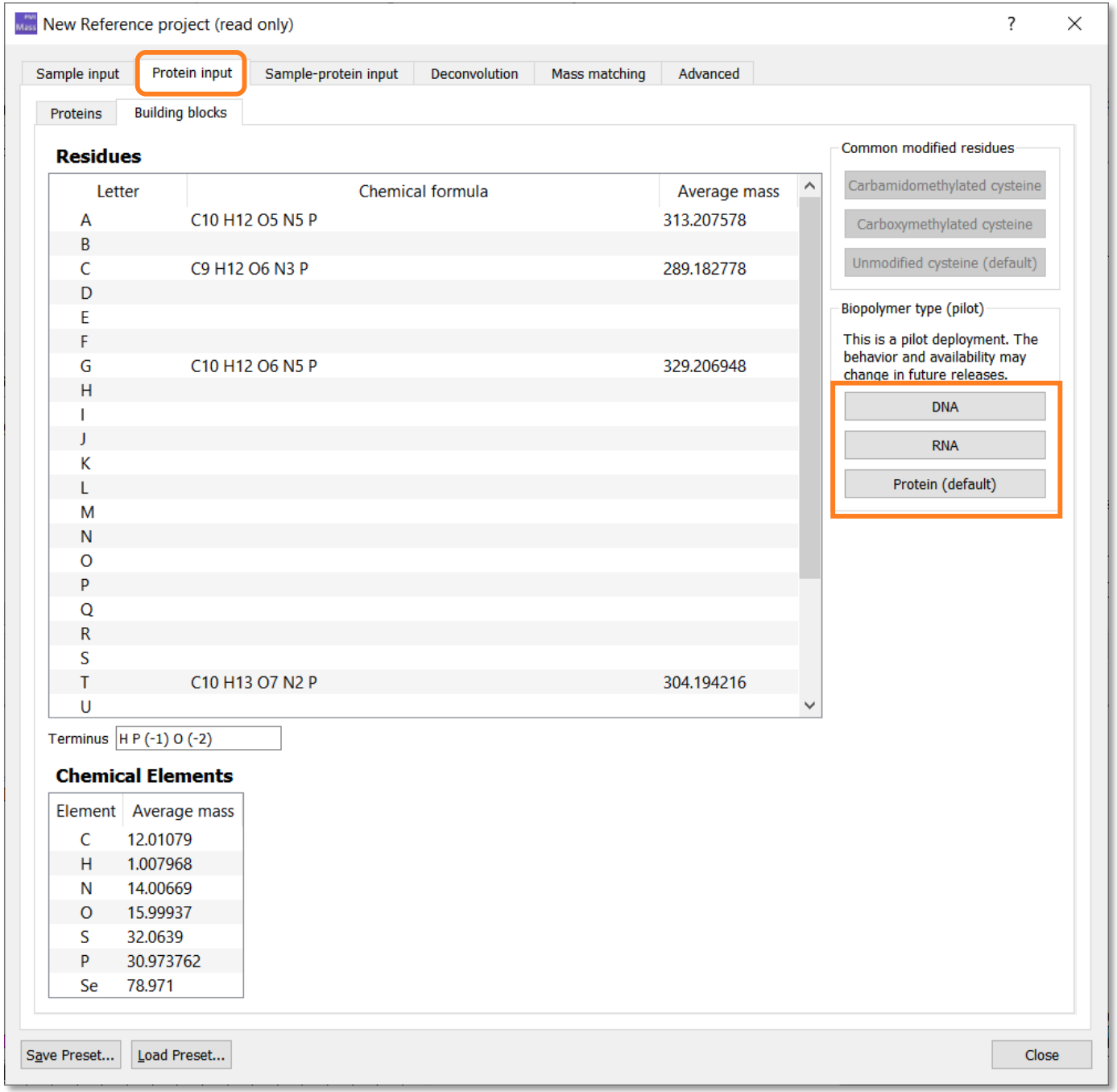
New Project



PROTEIN METRICS

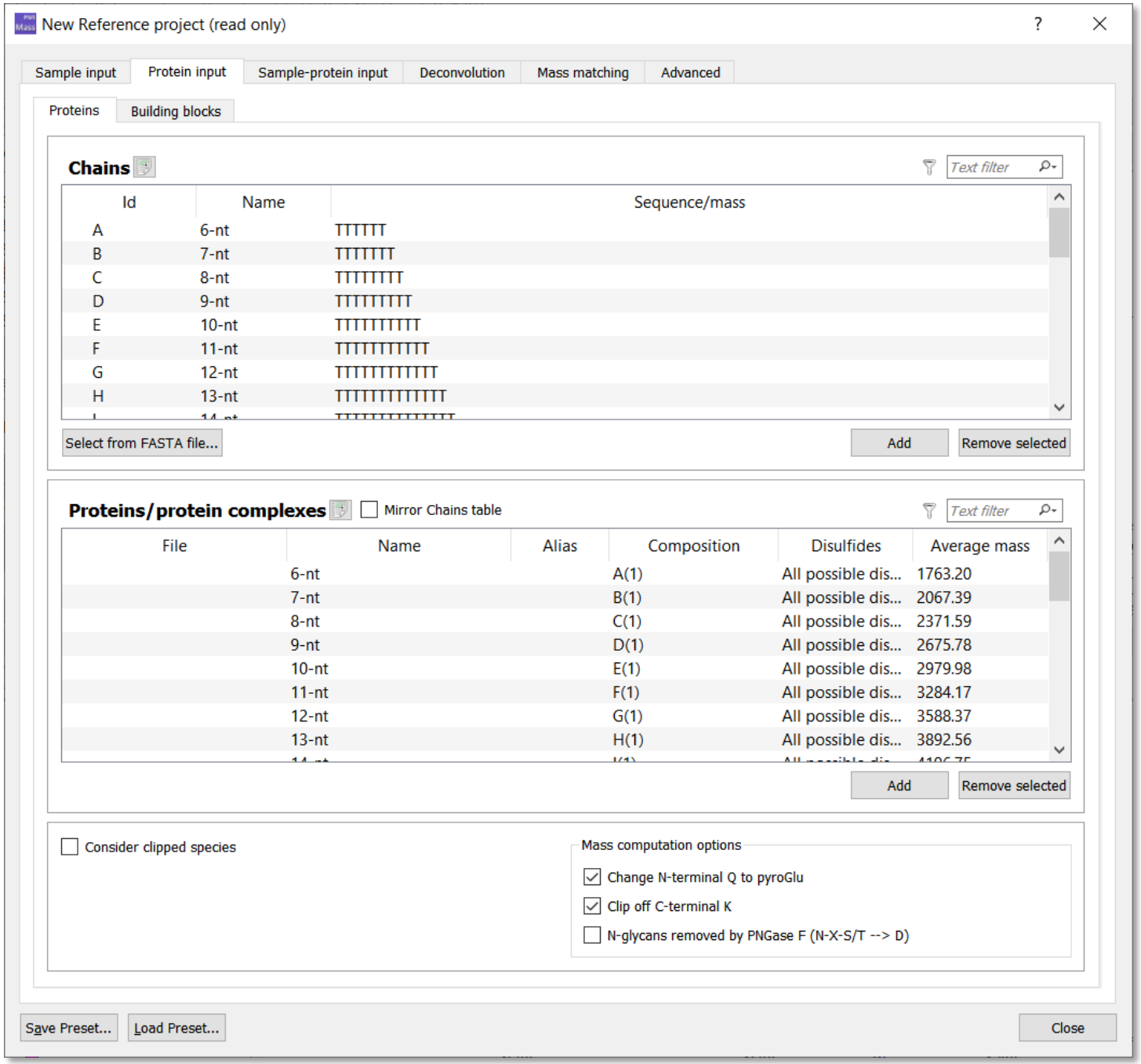
Define one-letter code for oligo sequences

- Presets for natural nucleotides
- Type in atomic formula for morpholinos, “locked” nucleic acids, thiophosphate, etc.



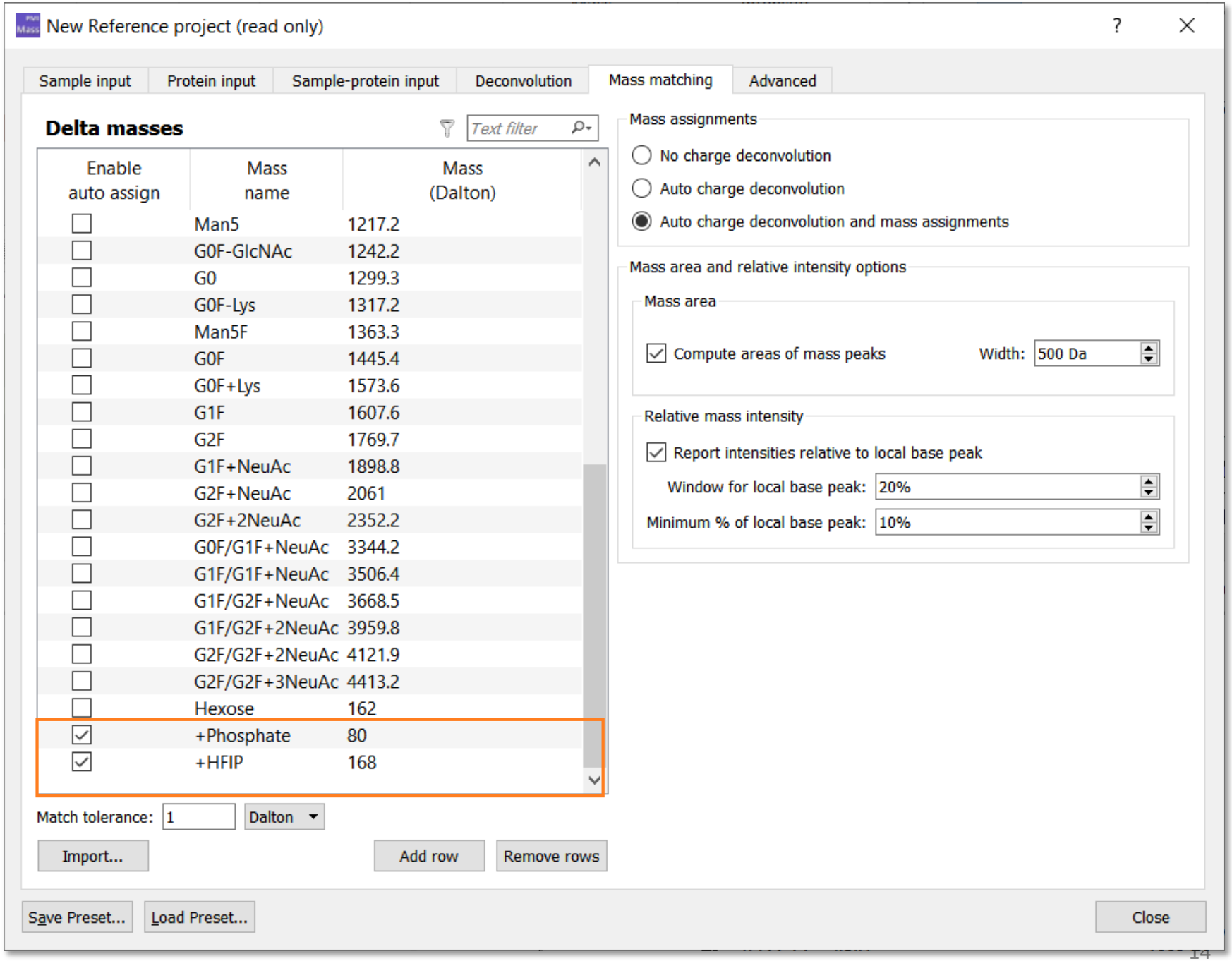
PROTEIN METRICS

Input sequences for mass matching

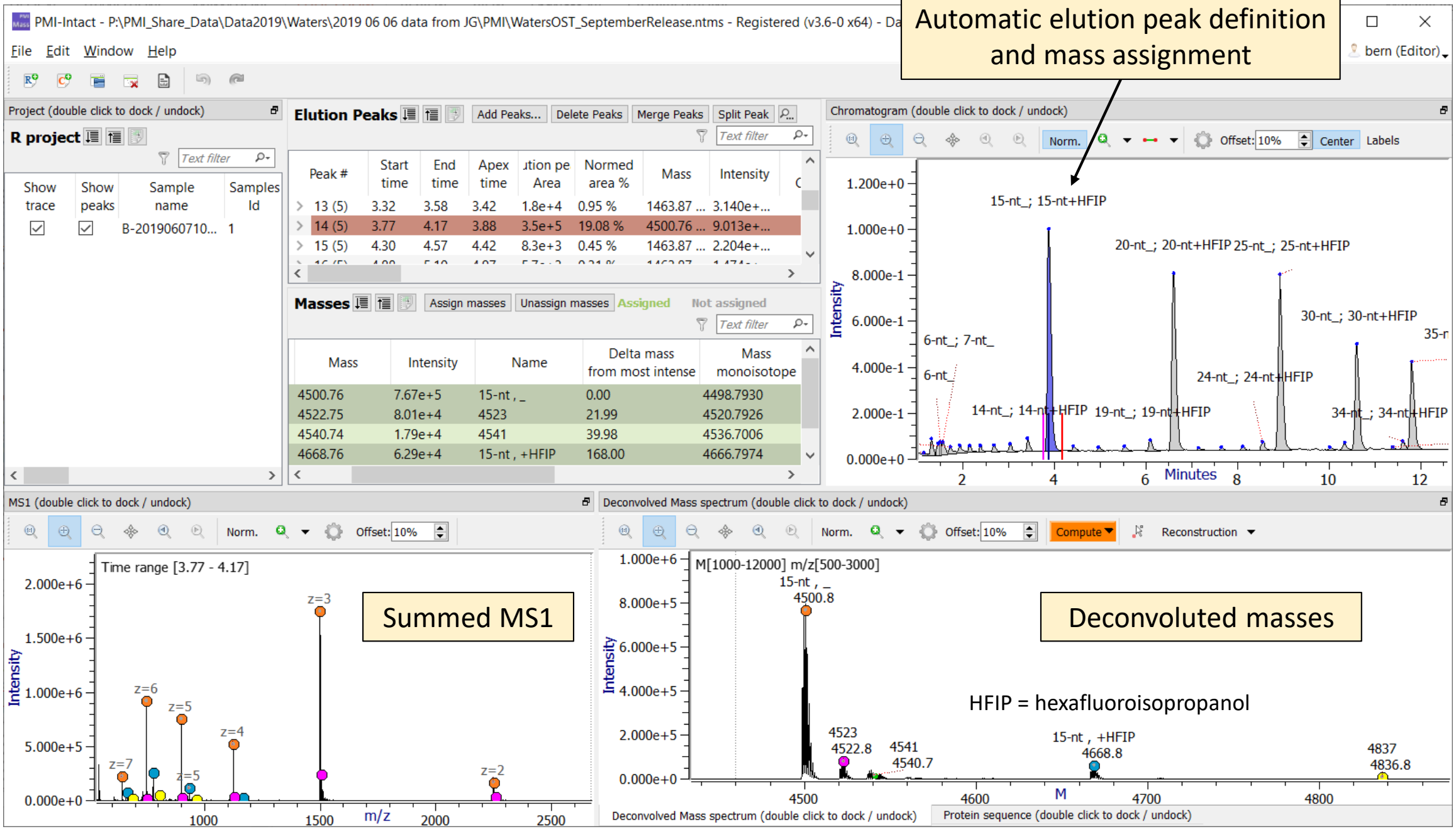


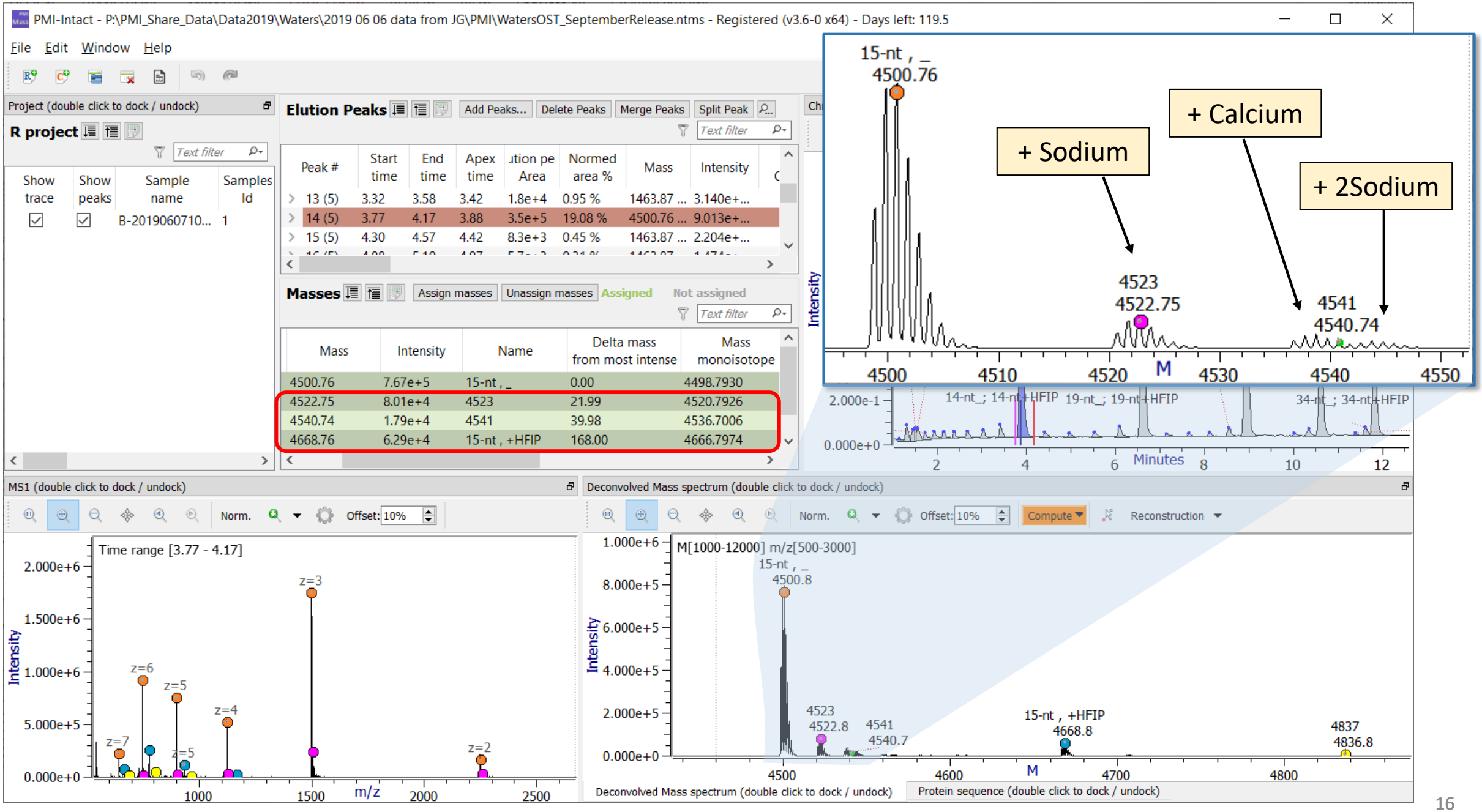
PROTEIN METRICS

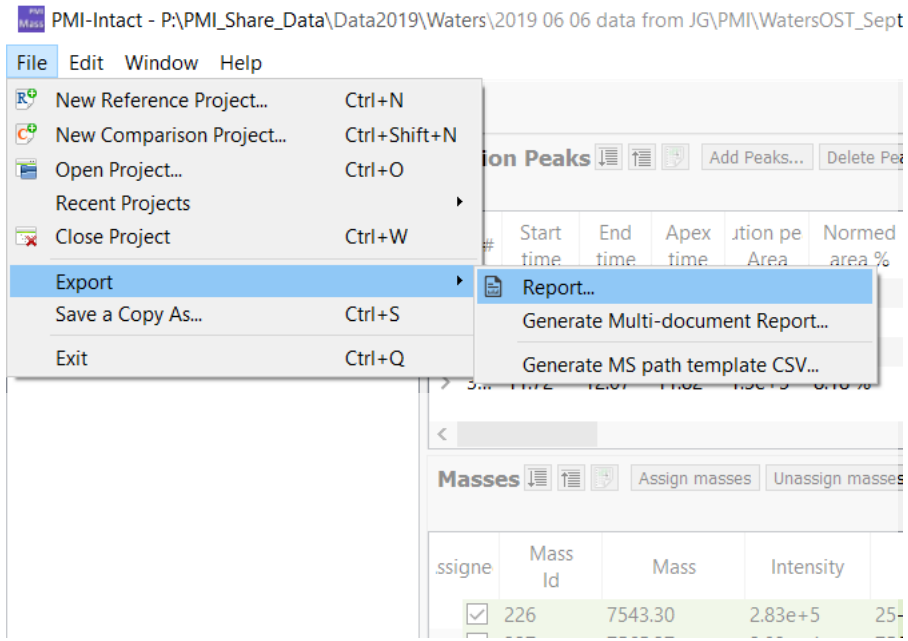
Define delta masses for impurities



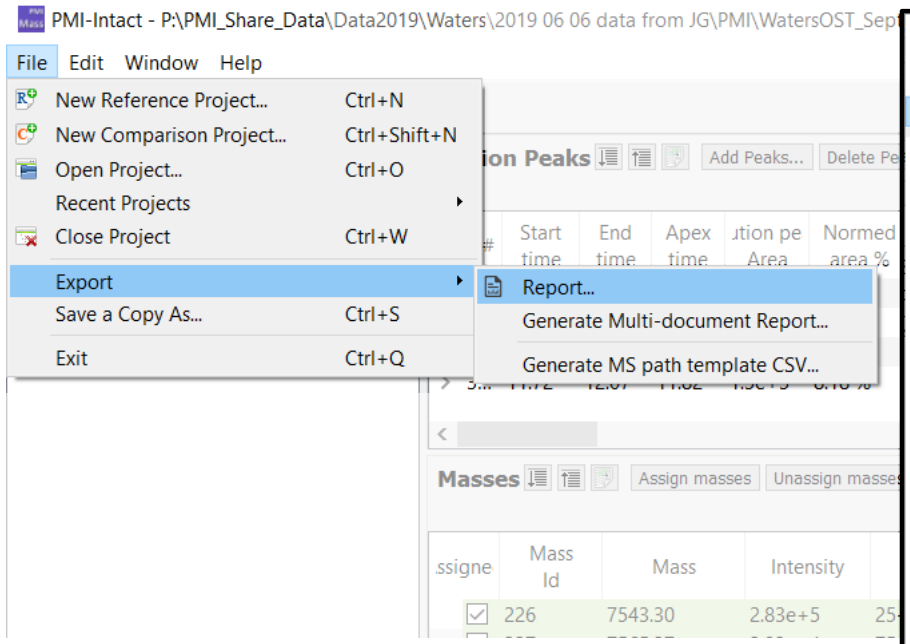
PROTEIN METRICS


Automatic elution peak definition and mass assignment
PMI-Intact - P:\PMI_Share_Data\Data2019\Waters\2019 06 06 data from JG\PMI\WatersOST_SeptemberRelease.ntms - Registered (v3.6-0 x64) - Da
File Edit Window Help
bern (Editor)
Project (double click to dock / undock)
R project
Text filter
Show trace
Show peaks
Sample name
Samples Id
B-2019060710...
1
Elution Peaks
Add Peaks...
Delete Peaks
Merge Peaks
Split Peak
Peak #
Start time
End time
Apex time
Area
Normed area %
Mass
Intensity
13 (5) 3.32 3.58 3.42 1.8e+4 0.95 % 1463.87 ... 3.140e+...
14 (5) 3.77 4.17 3.88 3.5e+5 19.08 % 4500.76 ... 9.013e+...
15 (5) 4.30 4.57 4.42 8.3e+3 0.45 % 1463.87 ... 2.204e+...
Masses
Assign masses
Unassign masses
Assigned
Not assigned
Mass
Intensity
Name
Delta mass from most intense
Mass monoisotope
4500.76 7.67e+5 15-nt , _ 0.00 4498.7930
4522.75 8.01e+4 4523 21.99 4520.7926
4540.74 1.79e+4 4541 39.98 4536.7006
4668.76 6.29e+4 15-nt , +HFIP 168.00 4666.7974
Chromatogram (double click to dock / undock)
Norm.
Offset: 10%
Center
Labels
Intensity
Minutes
15-nt_; 15-nt+HFIP
20-nt_; 20-nt+HFIP
25-nt_; 25-nt+HFIP
30-nt_; 30-nt+HFIP
24-nt_; 24-nt+HFIP
14-nt_; 14-nt+HFIP
19-nt_; 19-nt+HFIP
34-nt_; 34-nt+HFIP
6-nt_; 7-nt_
6-nt_
MS1 (double click to dock / undock)
Time range [3.77 - 4.17]
Summed MS1
Intensity
m/z
z=3
z=6
z=5
z=4
z=7
z=2
Deconvolved Mass spectrum (double click to dock / undock)
Compute
Reconstruction
M[1000-12000] m/z[500-3000]
15-nt , _
4500.8
4523
4522.8
4541
4540.7
15-nt , +HFIP
4668.8
4837
4836.8
Deconvoluted masses
HFIP = hexafluoroisopropanol
M
Deconvolved Mass spectrum (double click to dock / undock)
Protein sequence (double click to dock / undock)

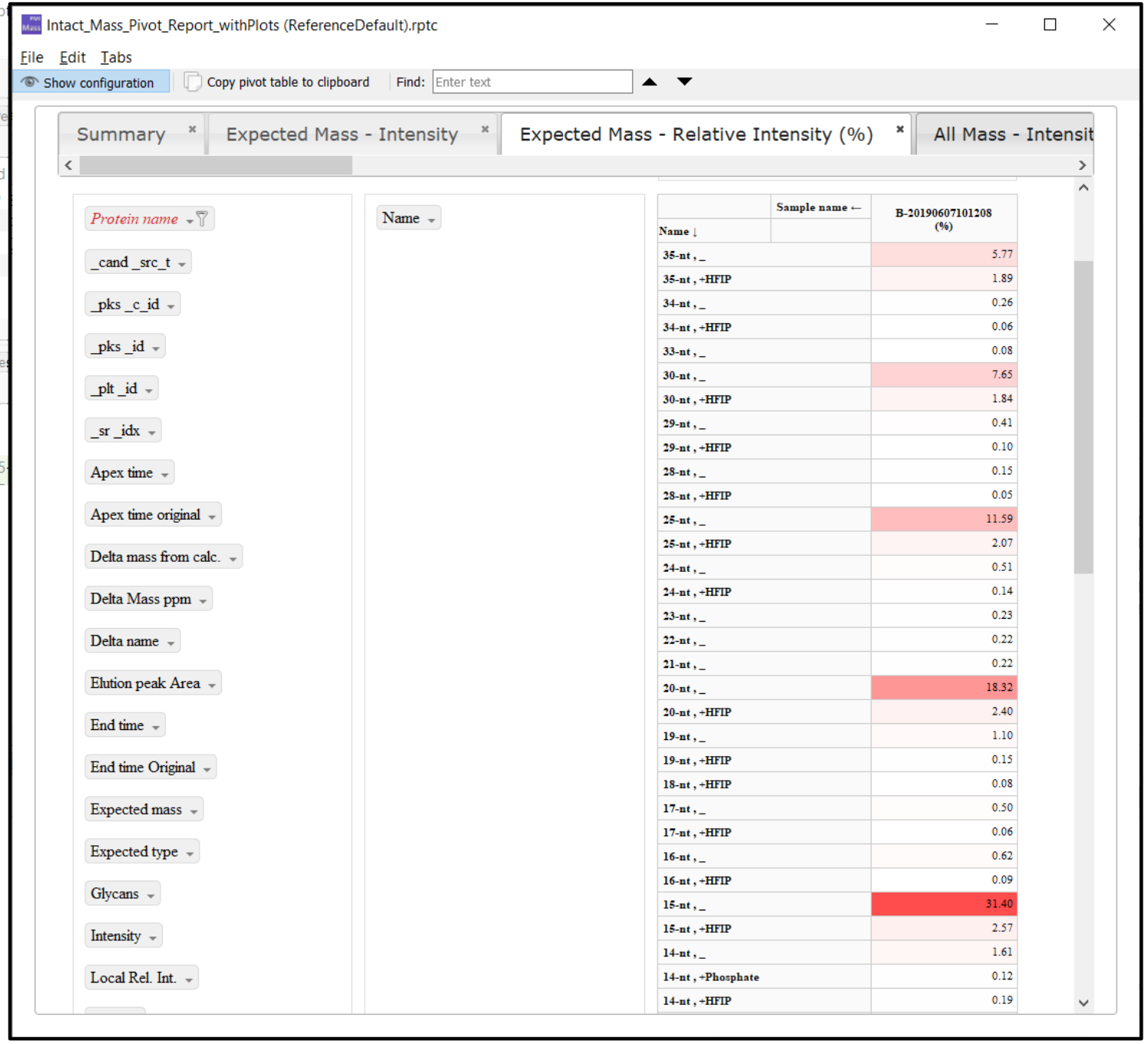

PMI-Intact - P:\PMI_Share_Data\Data2019\Waters\2019 06 06 data from JG\PMI\WatersOST_SeptemberRelease.ntms - Registered (v3.6-0 x64) - Days left: 119.5
File Edit Window Help
Project (double click to dock / undock)
R project
Show trace
Show peaks
Sample name
Samples Id
B-2019060710...
Elution Peaks
Add Peaks...
Delete Peaks
Merge Peaks
Split Peak
Peak #
Start time
End time
Apex time
Normed area %
Mass
Intensity
13 (5) 3.32 3.58 3.42 1.8e+4 0.95 % 1463.87 ... 3.140e+...
14 (5) 3.77 4.17 3.88 3.5e+5 19.08 % 4500.76 ... 9.013e+...
15 (5) 4.30 4.57 4.42 8.3e+3 0.45 % 1463.87 ... 2.204e+...
Masses
Assign masses
Unassign masses
Assigned
Not assigned
Mass
Intensity
Name
Delta mass from most intense
Mass monoisotope
4500.76 7.67e+5 15-nt , _ 0.00 4498.7930
4522.75 8.01e+4 4523 21.99 4520.7926
4540.74 1.79e+4 4541 39.98 4536.7006
4668.76 6.29e+4 15-nt , +HFIP 168.00 4666.7974
15-nt , _
4500.76
+ Sodium
+ Calcium
+ 2Sodium
4523
4522.75
4541
4540.74
Intensity
M
14-nt_; 14-nt+HFIP
19-nt_; 19-nt+HFIP
34-nt_; 34-nt+HFIP
Minutes
MS1 (double click to dock / undock)
Norm.
Offset: 10%
Time range [3.77 - 4.17]
z=3
z=6
z=5
z=4
z=7
z=2
m/z
Deconvolved Mass spectrum (double click to dock / undock)
Compute
Reconstruction
M[1000-12000] m/z[500-3000]
15-nt , _
4500.8
4523
4522.8
4541
4540.7
15-nt , +HFIP
4668.8
4837
4836.8
Protein sequence (double click to dock / undock)

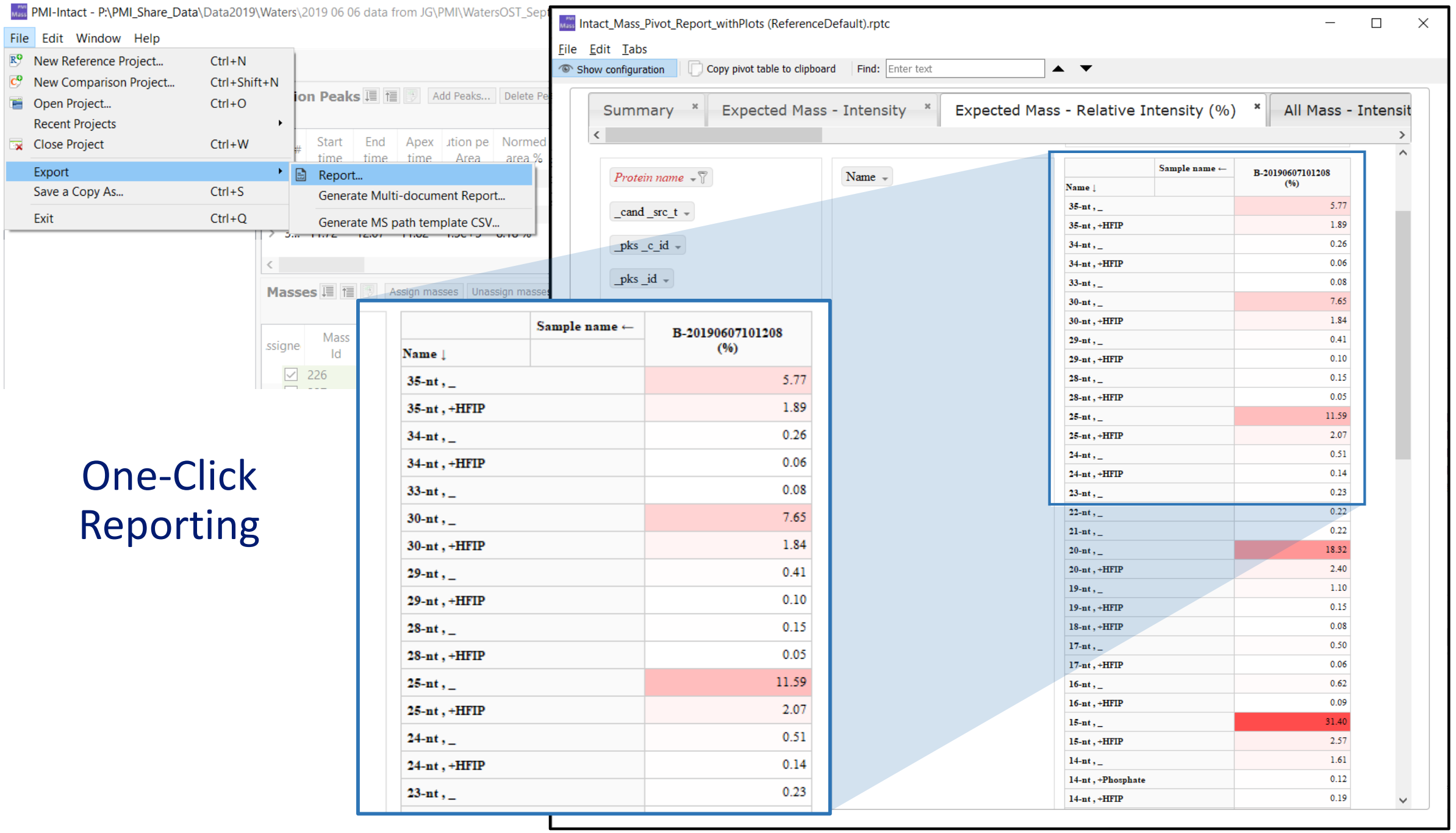


One-Click Reporting



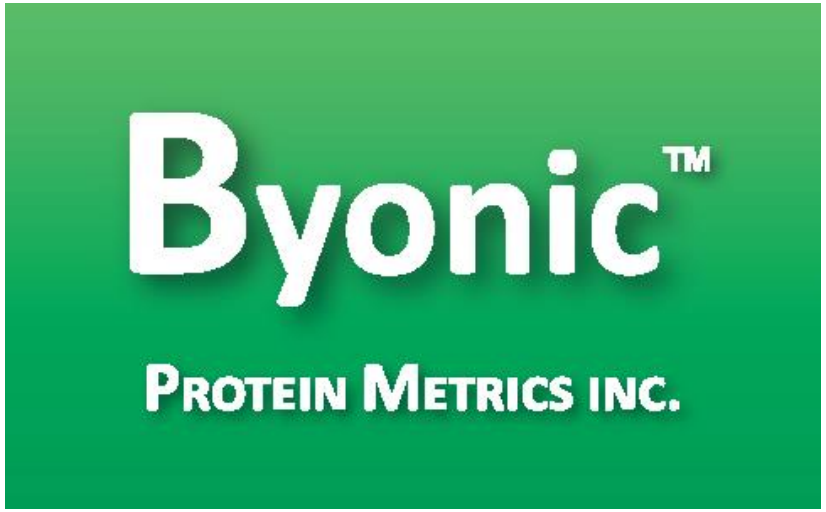
One-Click Reporting





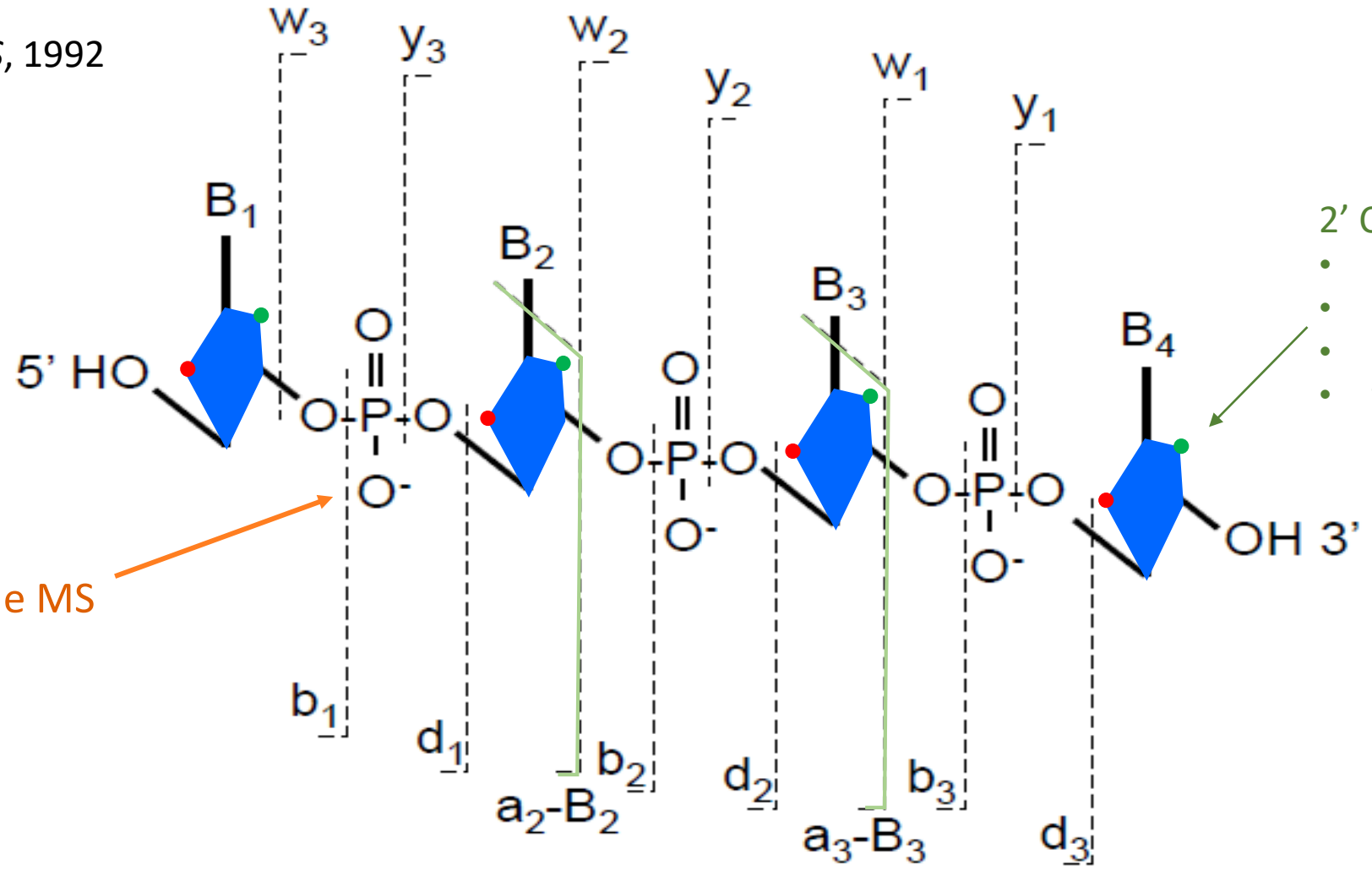
One-Click Reporting

Sequence Confirmation with MS2



Oligo Fragmentation

McLuckey et al, *JASMS*, 1992



Negative mode MS

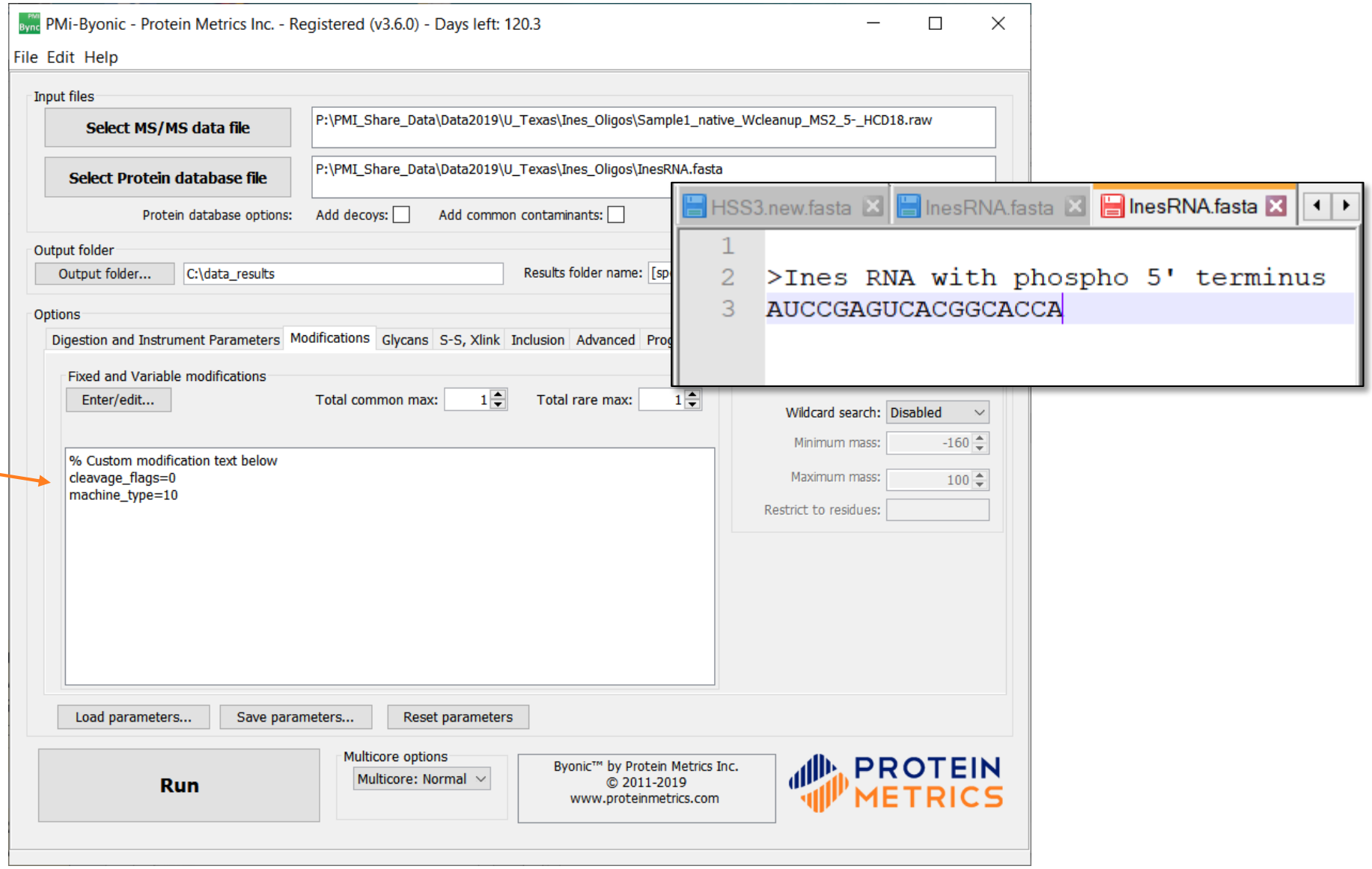
2' Carbon

- Deoxy
- Methyl
- Methoxyethyl
- "Locked"

PROTEIN METRICS

Byonic Parameters

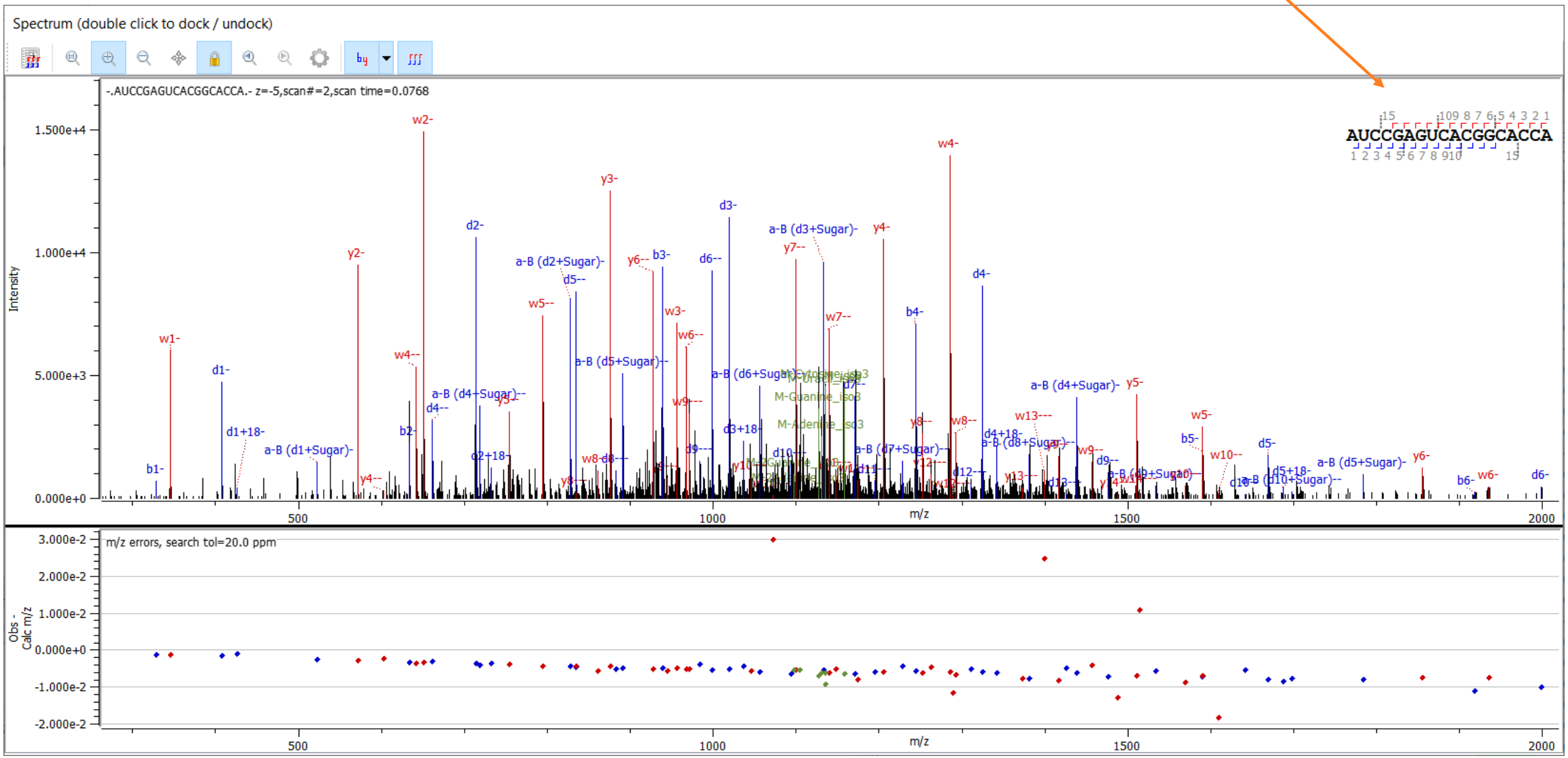
Text commands for experimental features



Synthetic RNA with HCD fragmentation

(Brodbelt Lab, U. Texas)

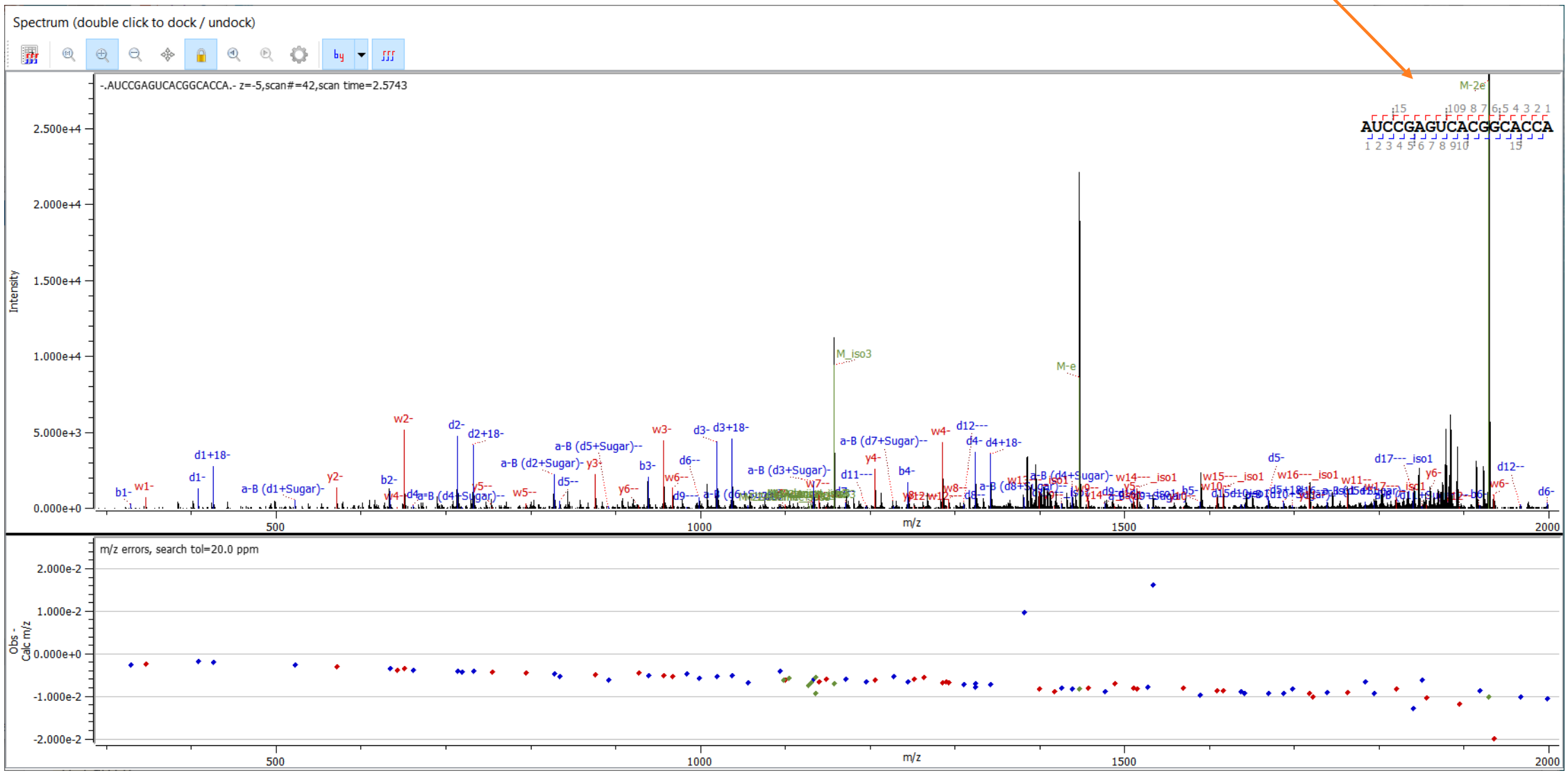
100% Sequence Coverage



UVPD fragmentation

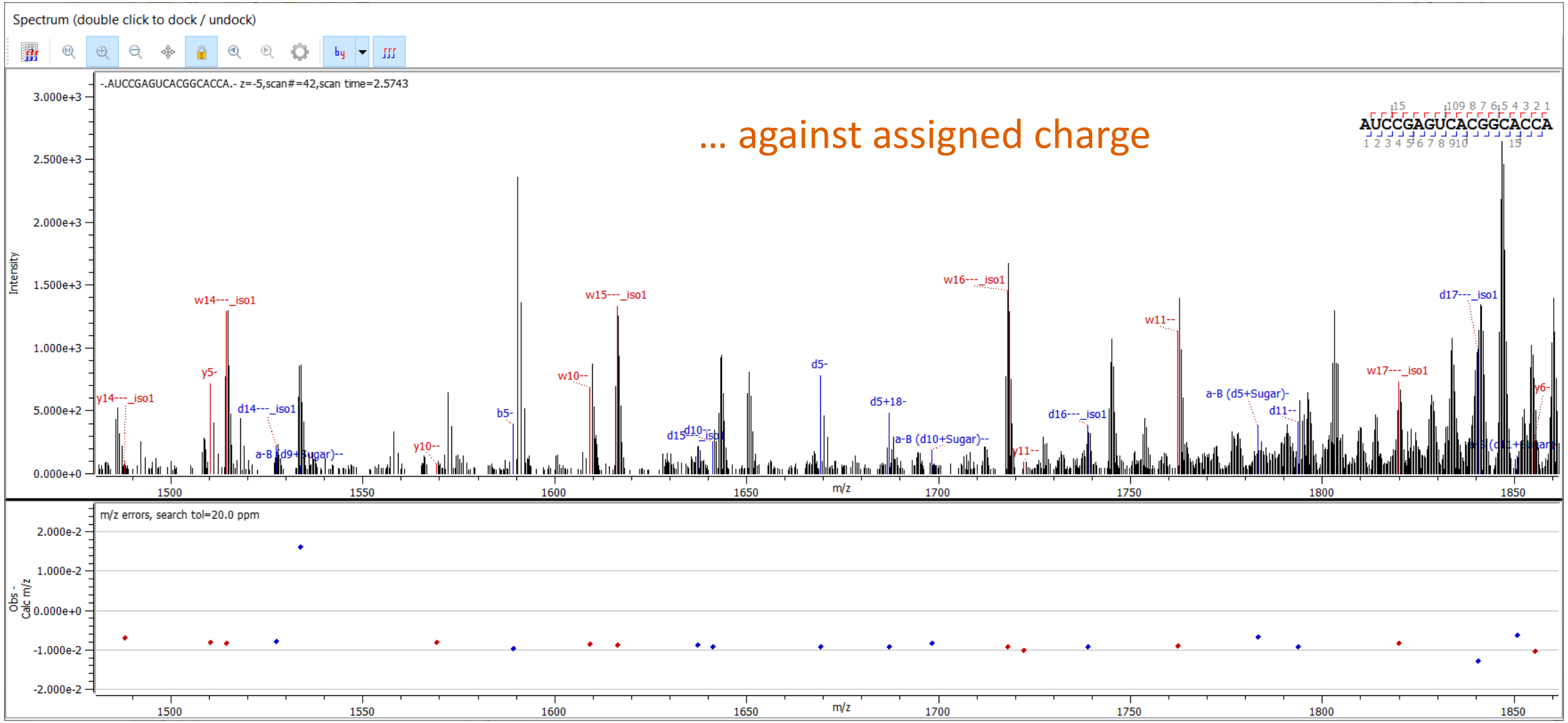
(Brodbelt Lab, U. Texas)

100% from both ends !



PROTEIN METRICS

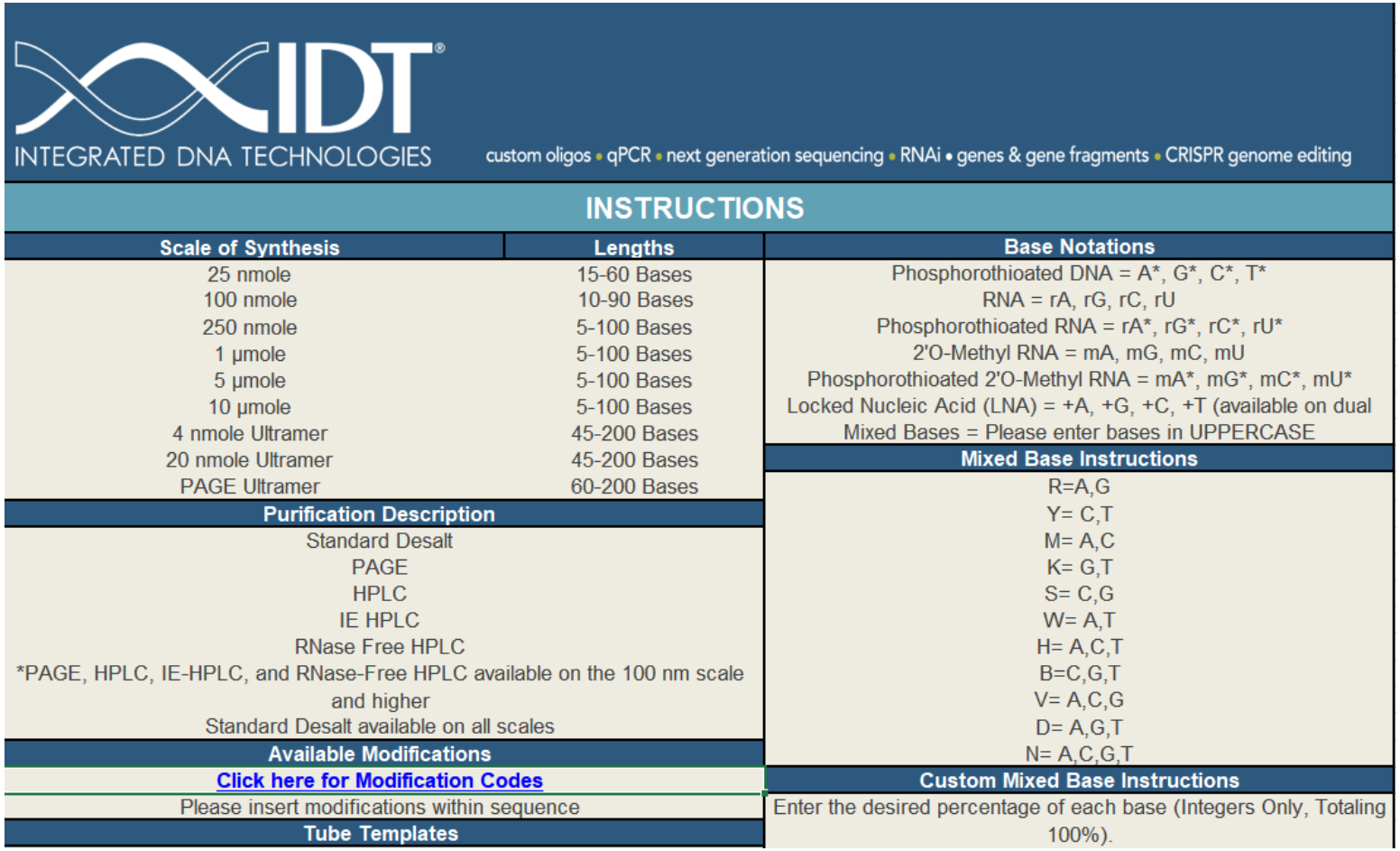
Byonic annotations for high-res MS2 check isotope peak spacing …



PROTEIN METRICS

Future Work

- Oligo-centric GUI
- Oligo-centric reporting
- Support for IDT codes
- Mixed protein / oligo (Crosslinked?!)



IDT®
INTEGRATED DNA TECHNOLOGIES
custom oligos • qPCR • next generation sequencing • RNAi • genes & gene fragments • CRISPR genome editing

INSTRUCTIONS

Scale of Synthesis	Lengths
25 nmole	15-60 Bases
100 nmole	10-90 Bases
250 nmole	5-100 Bases
1 µmole	5-100 Bases
5 µmole	5-100 Bases
10 µmole	5-100 Bases
4 nmole Ultramer	45-200 Bases
20 nmole Ultramer	45-200 Bases
PAGE Ultramer	60-200 Bases

Purification Description

Standard Desalt
PAGE
HPLC
IE HPLC
RNase Free HPLC
*PAGE, HPLC, IE-HPLC, and RNase-Free HPLC available on the 100 nm scale and higher
Standard Desalt available on all scales

Available Modifications

Click here for Modification Codes
Please insert modifications within sequence

Tube Templates

Base Notations

Phosphorothioated DNA = A*, G*, C*, T*
RNA = rA, rG, rC, rU
Phosphorothioated RNA = rA*, rG*, rC*, rU*
2'O-Methyl RNA = mA, mG, mC, mU
Phosphorothioated 2'O-Methyl RNA = mA*, mG*, mC*, mU*
Locked Nucleic Acid (LNA) = +A, +G, +C, +T (available on dual
Mixed Bases = Please enter bases in UPPERCASE

Mixed Base Instructions

R=A,G
Y= C,T
M= A,C
K= G,T
S= C,G
W= A,T
H= A,C,T
B=C,G,T
V= A,C,G
D= A,G,T
N= A,C,G,T

Custom Mixed Base Instructions

Enter the desired percentage of each base (Integers Only, Totaling 100%).

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For a **live demo** contact **info@proteinmetrics.com**

We're hiring!