Advanced Strategies for Proton Transfer Reactions Coupled with Parallel Ion Parking on a 21 T FT-ICR MS for Intact Protein Analysis

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Supplemental Figures Description

Figure S1-S3 Direct-infusion of apomyoglobin subject to PTR-MS[1]. These data illustrate that this experiment is stable over long-periods, such that it would be amenable to usage during LC-MS analysis. The mass spectrum (bottom inset) in each of the three figures shows the PTR products at the beginning (1), middle (2), and end (3) of a 74 min. acquisition period.

Figure S4 Statistical analysis of the base peak ($[M+12H]^{12+}$; apomyoglobin) observed during the experiment outlined in Supplemental Figure 1-3. The histogram of ion intensities is roughly Gaussian and is shown to have a %RSD = 19.7.

Figure S5A-B Fragment ion maps of generated from the tandem MS spectra of protein AG $[M+50H]^{50+}$ under ETD (A) and ETD-PTR (B) conditions.

Figure S6 PTR-PIP tandem MS spectra obtained from the isolation of the apomyoglobin $[M+21H]^{21+}$. A) was obtained under normal PIP waveform conditions. B) was acquired under rapid PIP waveform conditions. The desired charge state for parking as the $[M+14H]^{14+}$.

Figure S7 Reaction progress plots which track the abundance of precursor ion (apomyoglobin $[M+21H]^{21+}$), charged reduced species, and the TIC during PTR under A) normal ion parking and B) rapid ion parking as a function of reaction time. These plots illustrate that while rapid ion parking proceed at 5-10x the reaction rate the reaction efficiency is unaffected. Because PTR consumes charge, undergoing 7 generations of PTR to park the $[M+14H]^{14+}$ imposes the maximum efficiency at 66% the signal abundance of the isolated precursor.

Figure S8 High resolution reagent ion spectra analyzed within the FT-ICR. These spectra were acquired after PIP waveforms were applied within the linear RF ion trap at A) 0.00, B) 0.020, and C) 0.065 reagent anion parking amplitudes. From these data, the loss of fluorine is evident within C) resultant from the elevated reagent anion parking amplitude.

Figure S9 A zoomed inset from Supplemental Figure 8C illustrating the mass loss is consistent with the loss of a single fluorine from the parent ion.

Figure S10 A plot of the intact reagent anion (PFMD) and the fluorine loss fragment (PFMD-F) as a function of reaction time under various reagent anion parking amplitudes. These data illustrate how the PFMD-F are temporally generated as PTR-PIP reactions proceed.

Figure S11 Reaction rate vs reagent parking amplitude. Regions labeled for their observed parking efficiency.

Figure S12 Reaction rate as determined from the precursor ion decay under normal ion parking, rapid ion parking, and uncontrolled PTR.

Figure S1

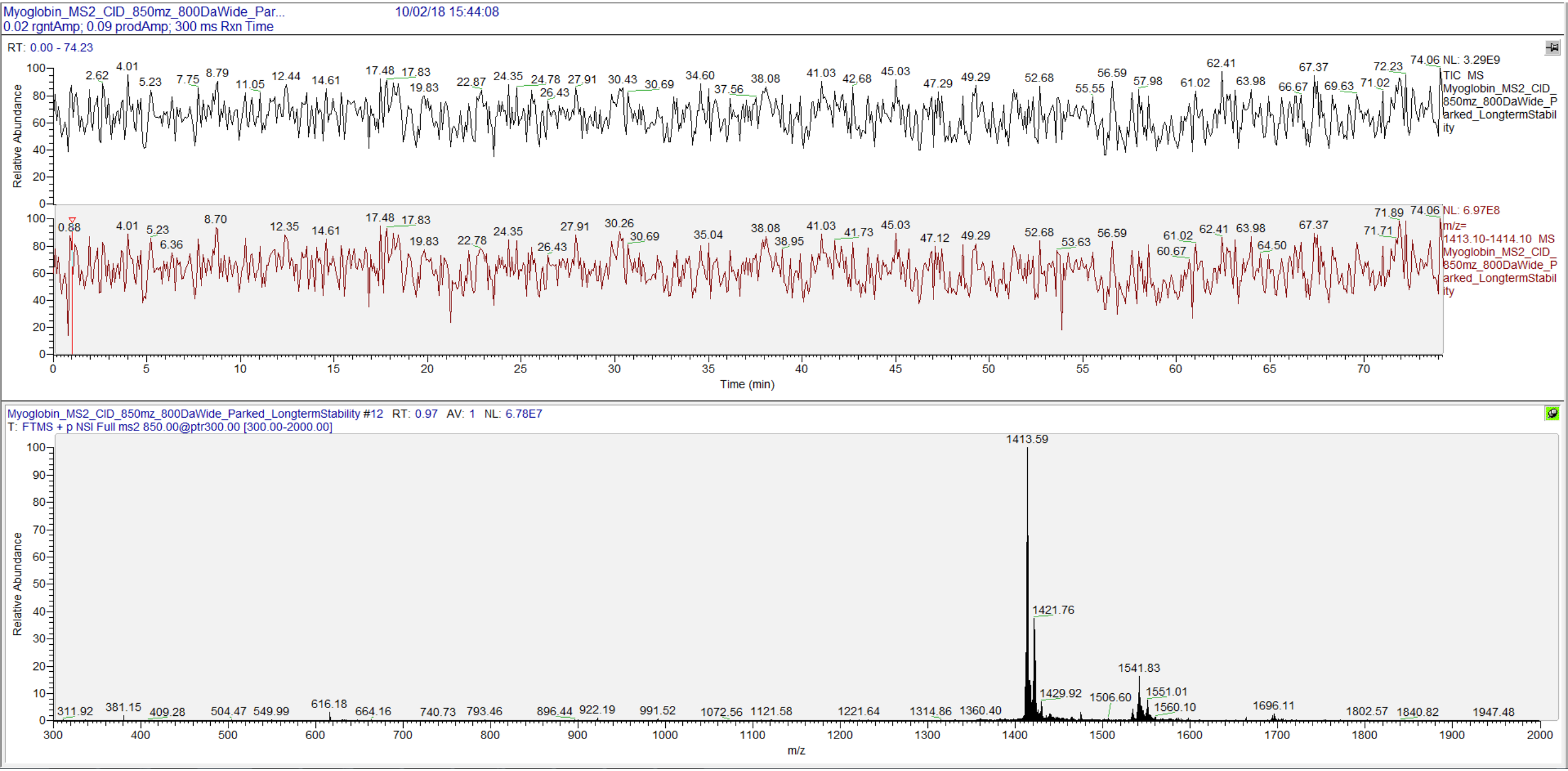

Myoglobin_MS2_CID_850mz_800DaWide_Par...
10/02/18 15:44:08
0.02 rgntAmp; 0.09 prodAmp; 300 ms Rxn Time
RT: 0.00 - 74.23
Relative Abundance
NL: 3.29E9
TIC MS
Myoglobin_MS2_CID_850mz_800DaWide_Parked_LongtermStability
NL: 6.97E8
m/z=
1413.10-1414.10 MS
Myoglobin_MS2_CID_850mz_800DaWide_Parked_LongtermStability
Time (min)
Myoglobin_MS2_CID_850mz_800DaWide_Parked_LongtermStability #12 RT: 0.97 AV: 1 NL: 6.78E7
T: FTMS + p NSI Full ms2 850.00@ptr300.00 [300.00-2000.00]
1413.59
1421.76
1541.83
1429.92
1506.60
1551.01
1560.10
1696.11
m/z

Figure S2

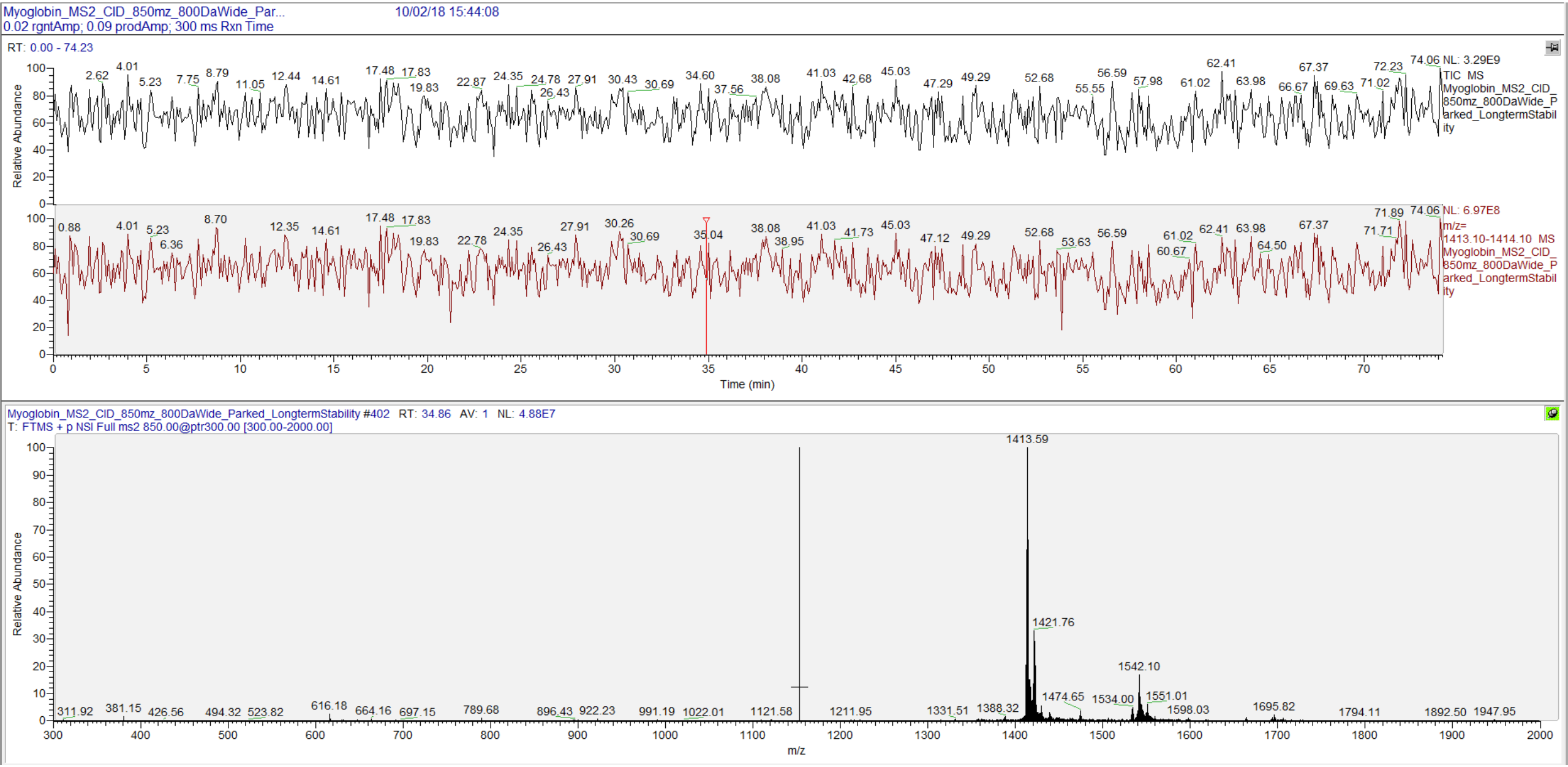

Myoglobin_MS2_CID_850mz_800DaWide_Par...
10/02/18 15:44:08
0.02 rgntAmp; 0.09 prodAmp; 300 ms Rxn Time
RT: 0.00 - 74.23
Relative Abundance
NL: 3.29E9
TIC MS
Myoglobin_MS2_CID_850mz_800DaWide_Parked_LongtermStability
NL: 6.97E8
m/z=
1413.10-1414.10 MS
Myoglobin_MS2_CID_850mz_800DaWide_Parked_LongtermStability
Time (min)
Myoglobin_MS2_CID_850mz_800DaWide_Parked_LongtermStability #402 RT: 34.86 AV: 1 NL: 4.88E7
T: FTMS + p NSI Full ms2 850.00@ptr300.00 [300.00-2000.00]
1413.59
1421.76
1542.10
m/z

Figure S3

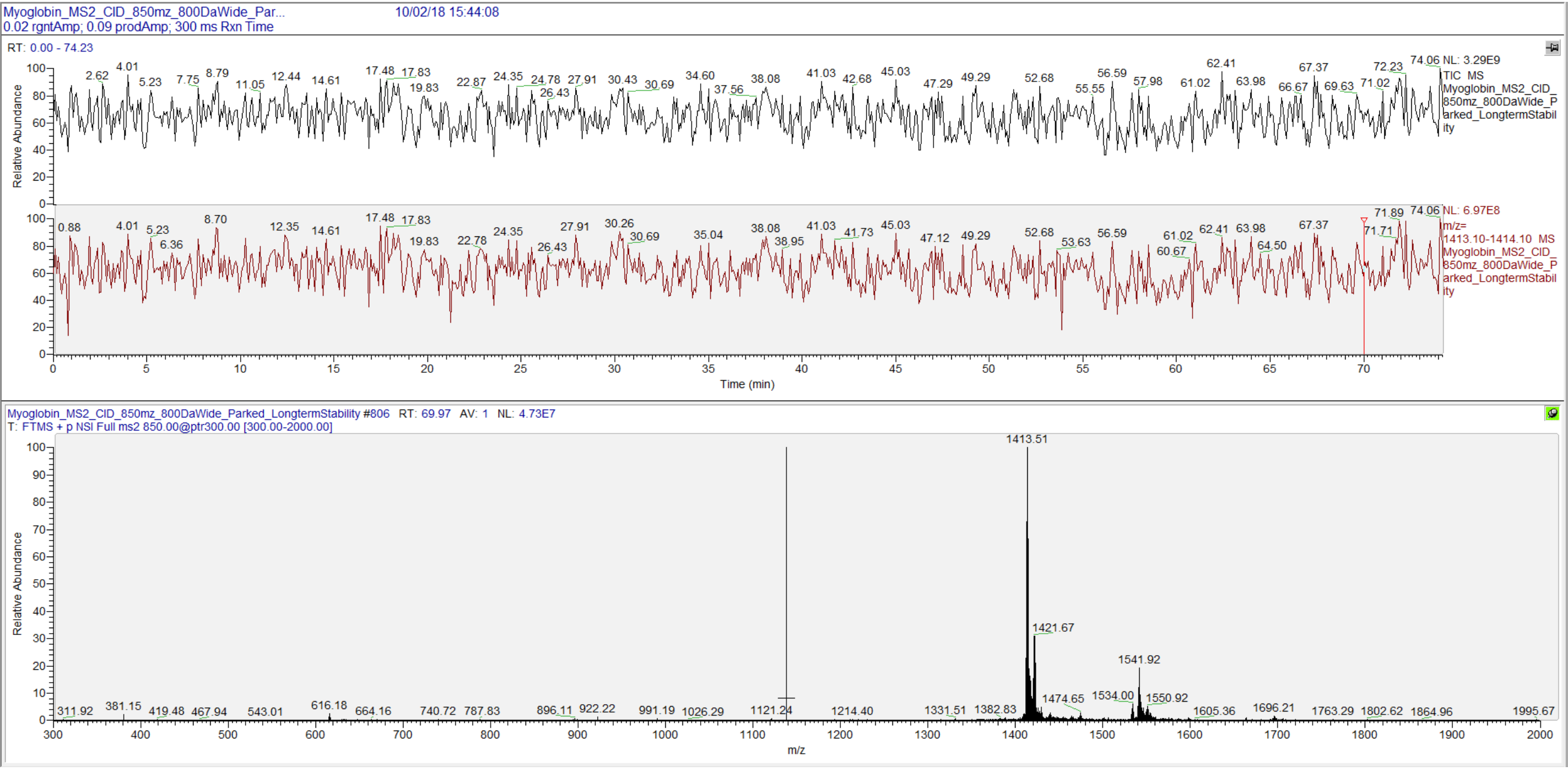

Myoglobin_MS2_CID_850mz_800DaWide_Par...
10/02/18 15:44:08
0.02 rgntAmp; 0.09 prodAmp; 300 ms Rxn Time
RT: 0.00 - 74.23
Relative Abundance
Time (min)
TIC MS Myoglobin_MS2_CID_850mz_800DaWide_Parked_LongtermStability
NL: 3.29E9
NL: 6.97E8
m/z= 1413.10-1414.10 MS Myoglobin_MS2_CID_850mz_800DaWide_Parked_LongtermStability
Myoglobin_MS2_CID_850mz_800DaWide_Parked_LongtermStability #806 RT: 69.97 AV: 1 NL: 4.73E7
T: FTMS + p NSI Full ms2 850.00@ptr300.00 [300.00-2000.00]
1413.51
1421.67
1541.92
m/z

Figure S4

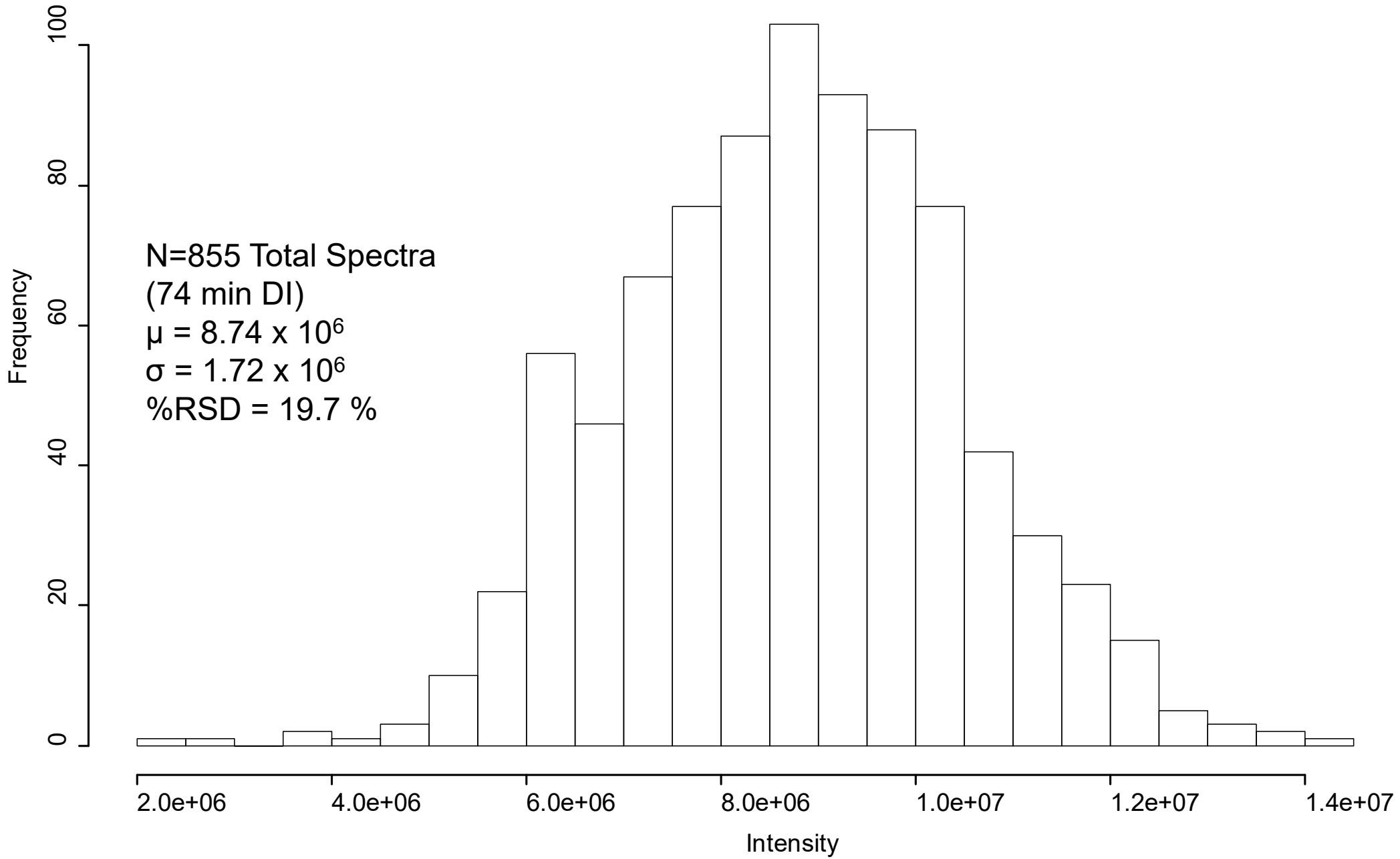

N=855 Total Spectra
(74 min DI)
μ = 8.74 x 10^6
σ = 1.72 x 10^6
%RSD = 19.7 %
Frequency
0
20
40
60
80
100
2.0e+06
4.0e+06
6.0e+06
8.0e+06
1.0e+07
1.2e+07
1.4e+07
Intensity

Figure S5

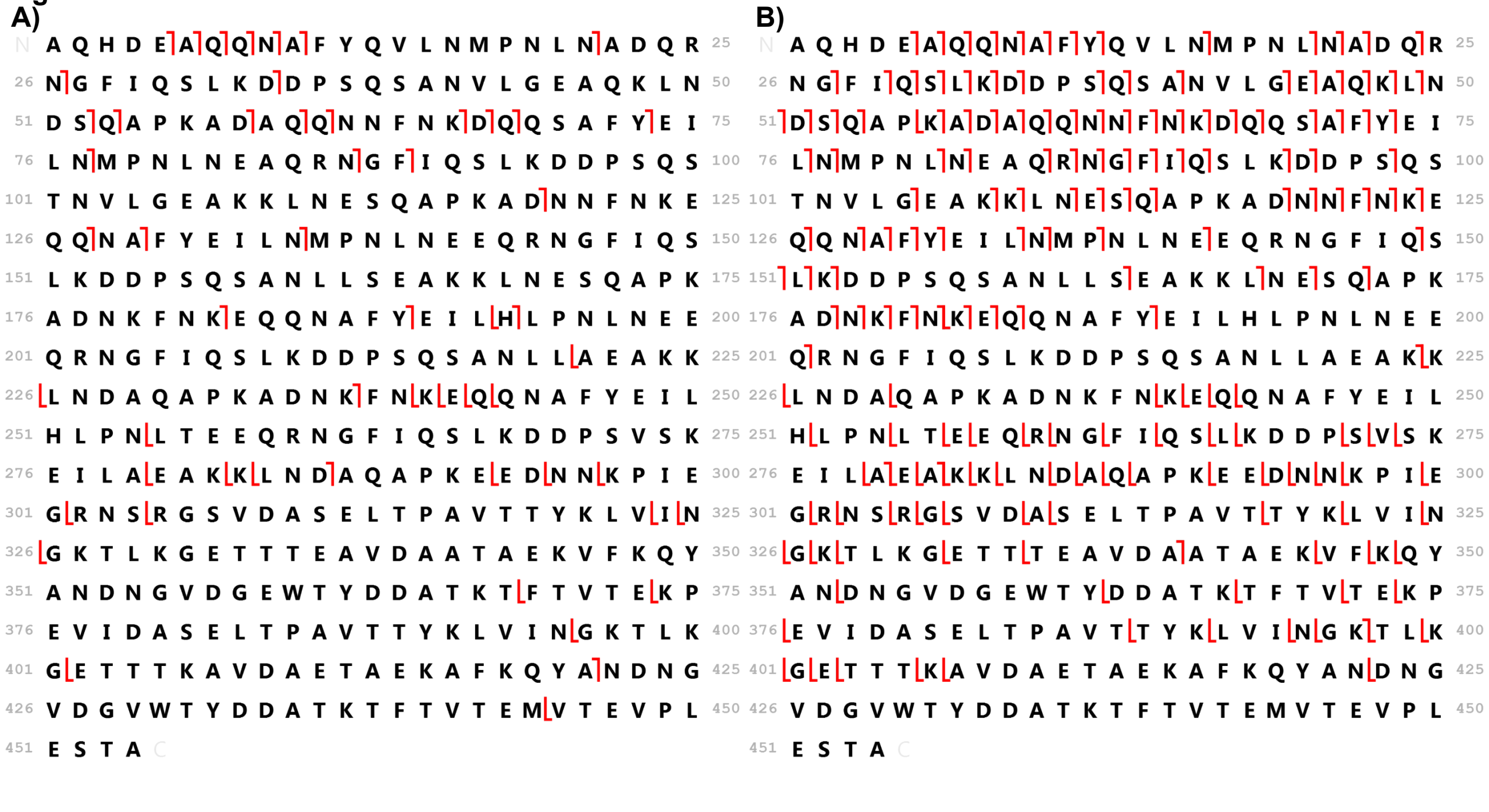

A)
N A Q H D E A Q Q N A F Y Q V L N M P N L N A D Q R 25
26 N G F I Q S L K D D P S Q S A N V L G E A Q K L N 50
51 D S Q A P K A D A Q Q N N F N K D Q Q S A F Y E I 75
76 L N M P N L N E A Q R N G F I Q S L K D D P S Q S 100
101 T N V L G E A K K L N E S Q A P K A D N N F N K E 125
126 Q Q N A F Y E I L N M P N L N E E Q R N G F I Q S 150
151 L K D D P S Q S A N L L S E A K K L N E S Q A P K 175
176 A D N K F N K E Q Q N A F Y E I L H L P N L N E E 200
201 Q R N G F I Q S L K D D P S Q S A N L L A E A K K 225
226 L N D A Q A P K A D N K F N K E Q Q N A F Y E I L 250
251 H L P N L T E E Q R N G F I Q S L K D D P S V S K 275
276 E I L A E A K K L N D A Q A P K E E D N N K P I E 300
301 G R N S R G S V D A S E L T P A V T T Y K L V I N 325
326 G K T L K G E T T T E A V D A A T A E K V F K Q Y 350
351 A N D N G V D G E W T Y D D A T K T F T V T E K P 375
376 E V I D A S E L T P A V T T Y K L V I N G K T L K 400
401 G E T T T K A V D A E T A E K A F K Q Y A N D N G 425
426 V D G V W T Y D D A T K T F T V T E M V T E V P L 450
451 E S T A C
B)
N A Q H D E A Q Q N A F Y Q V L N M P N L N A D Q R 25
26 N G F I Q S L K D D P S Q S A N V L G E A Q K L N 50
51 D S Q A P K A D A Q Q N N F N K D Q Q S A F Y E I 75
76 L N M P N L N E A Q R N G F I Q S L K D D P S Q S 100
101 T N V L G E A K K L N E S Q A P K A D N N F N K E 125
126 Q Q N A F Y E I L N M P N L N E E Q R N G F I Q S 150
151 L K D D P S Q S A N L L S E A K K L N E S Q A P K 175
176 A D N K F N K E Q Q N A F Y E I L H L P N L N E E 200
201 Q R N G F I Q S L K D D P S Q S A N L L A E A K K 225
226 L N D A Q A P K A D N K F N K E Q Q N A F Y E I L 250
251 H L P N L T E E Q R N G F I Q S L K D D P S V S K 275
276 E I L A E A K K L N D A Q A P K E E D N N K P I E 300
301 G R N S R G S V D A S E L T P A V T T Y K L V I N 325
326 G K T L K G E T T T E A V D A A T A E K V F K Q Y 350
351 A N D N G V D G E W T Y D D A T K T F T V T E K P 375
376 E V I D A S E L T P A V T T Y K L V I N G K T L K 400
401 G E T T T K A V D A E T A E K A F K Q Y A N D N G 425
426 V D G V W T Y D D A T K T F T V T E M V T E V P L 450
451 E S T A C

Figure S6

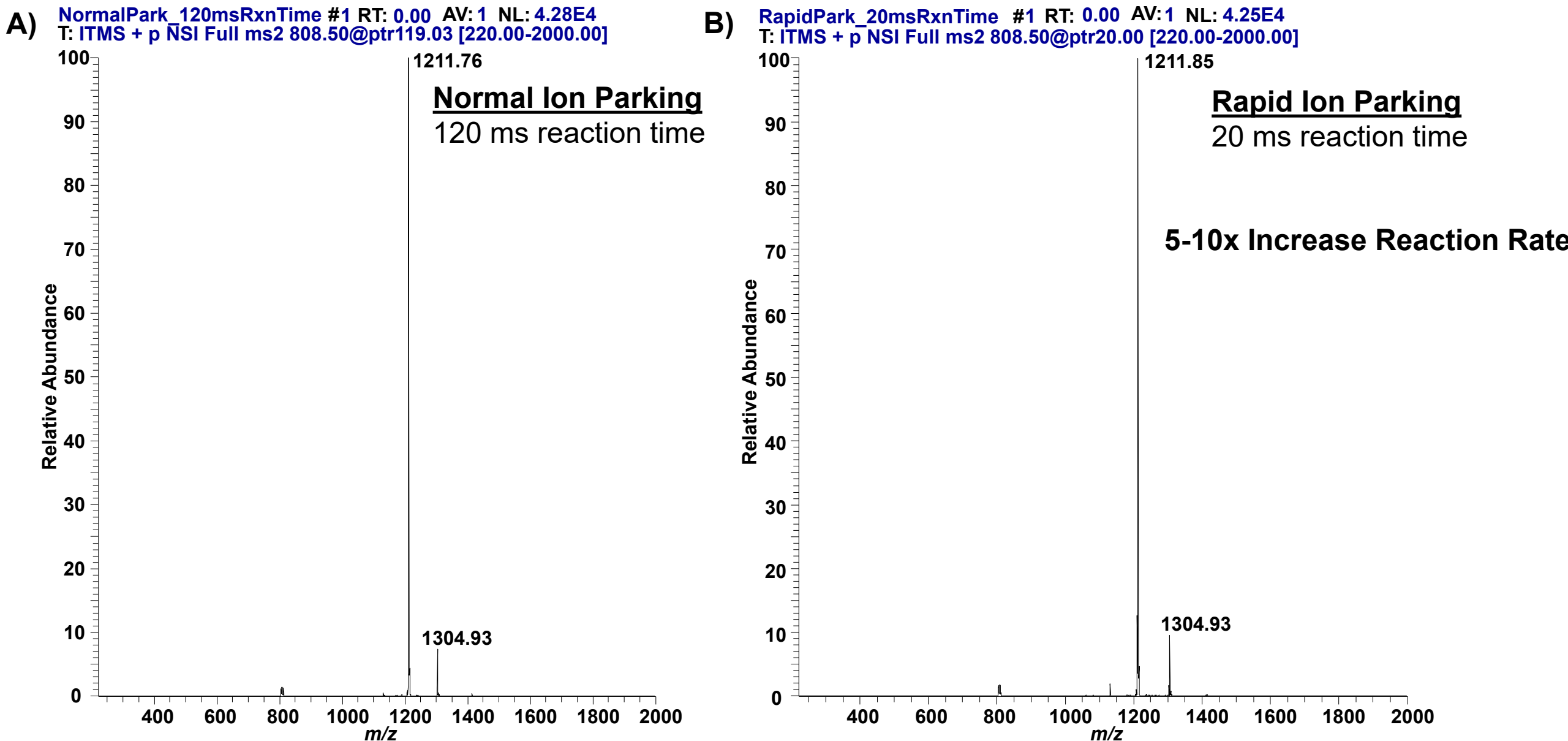
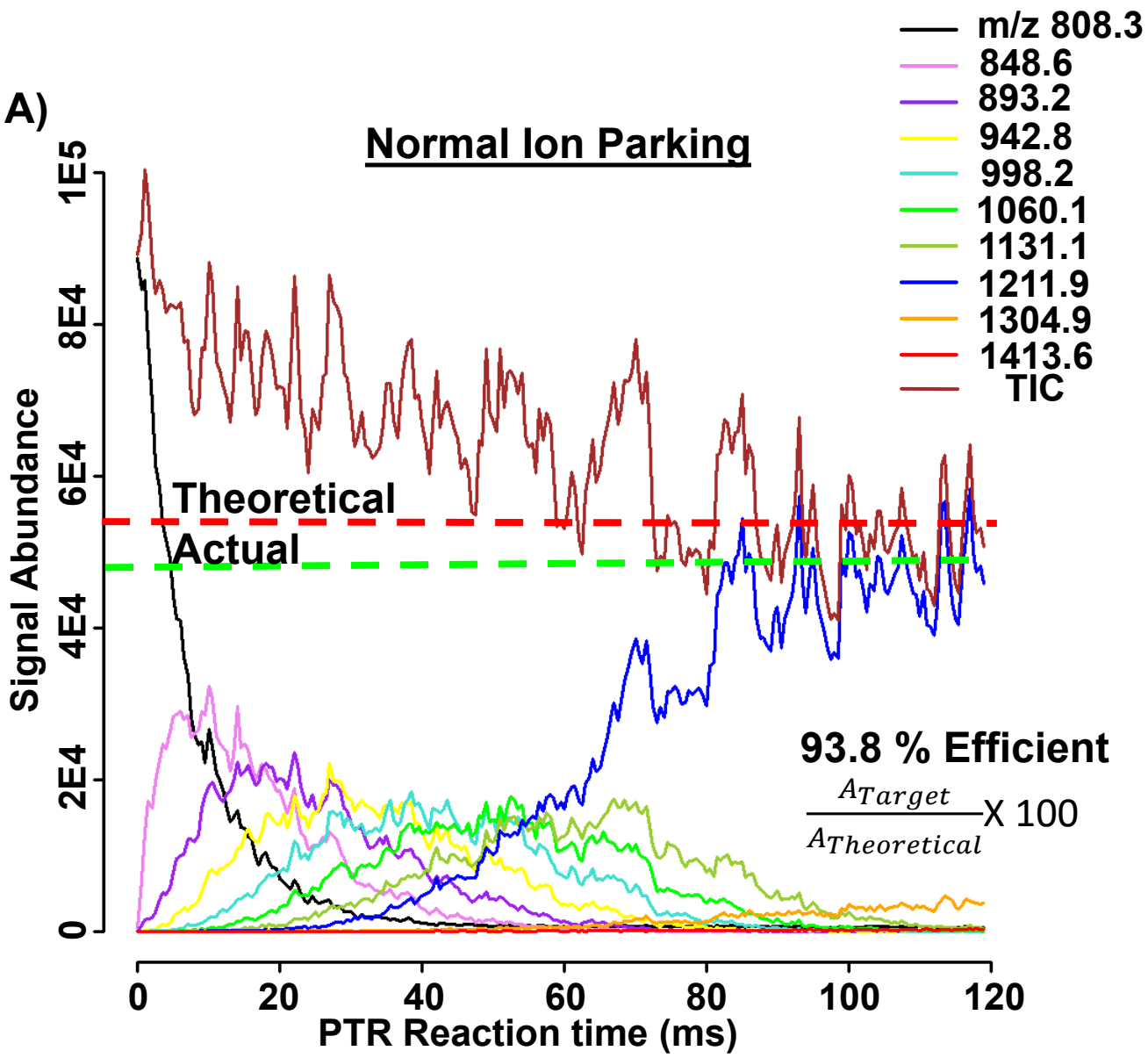

A)
NormalPark_120msRxnTime #1 RT: 0.00 AV: 1 NL: 4.28E4
T: ITMS + p NSI Full ms2 808.50@ptr119.03 [220.00-2000.00]
1211.76
Normal Ion Parking
120 ms reaction time
1304.93
Relative Abundance
m/z
B)
RapidPark_20msRxnTime #1 RT: 0.00 AV: 1 NL: 4.25E4
T: ITMS + p NSI Full ms2 808.50@ptr20.00 [220.00-2000.00]
1211.85
Rapid Ion Parking
20 ms reaction time
5-10x Increase Reaction Rate
1304.93
Relative Abundance
m/z

Figure S7


A)
Normal Ion Parking
m/z 808.3
848.6
893.2
942.8
998.2
1060.1
1131.1
1211.9
1304.9
1413.6
TIC
Theoretical
Actual
93.8 % Efficient
$\frac{A_{Target}}{A_{Theoretical}}$ X 100
Signal Abundance
0
2E4
4E4
6E4
8E4
1E5
0
20
40
60
80
100
120
PTR Reaction time (ms)

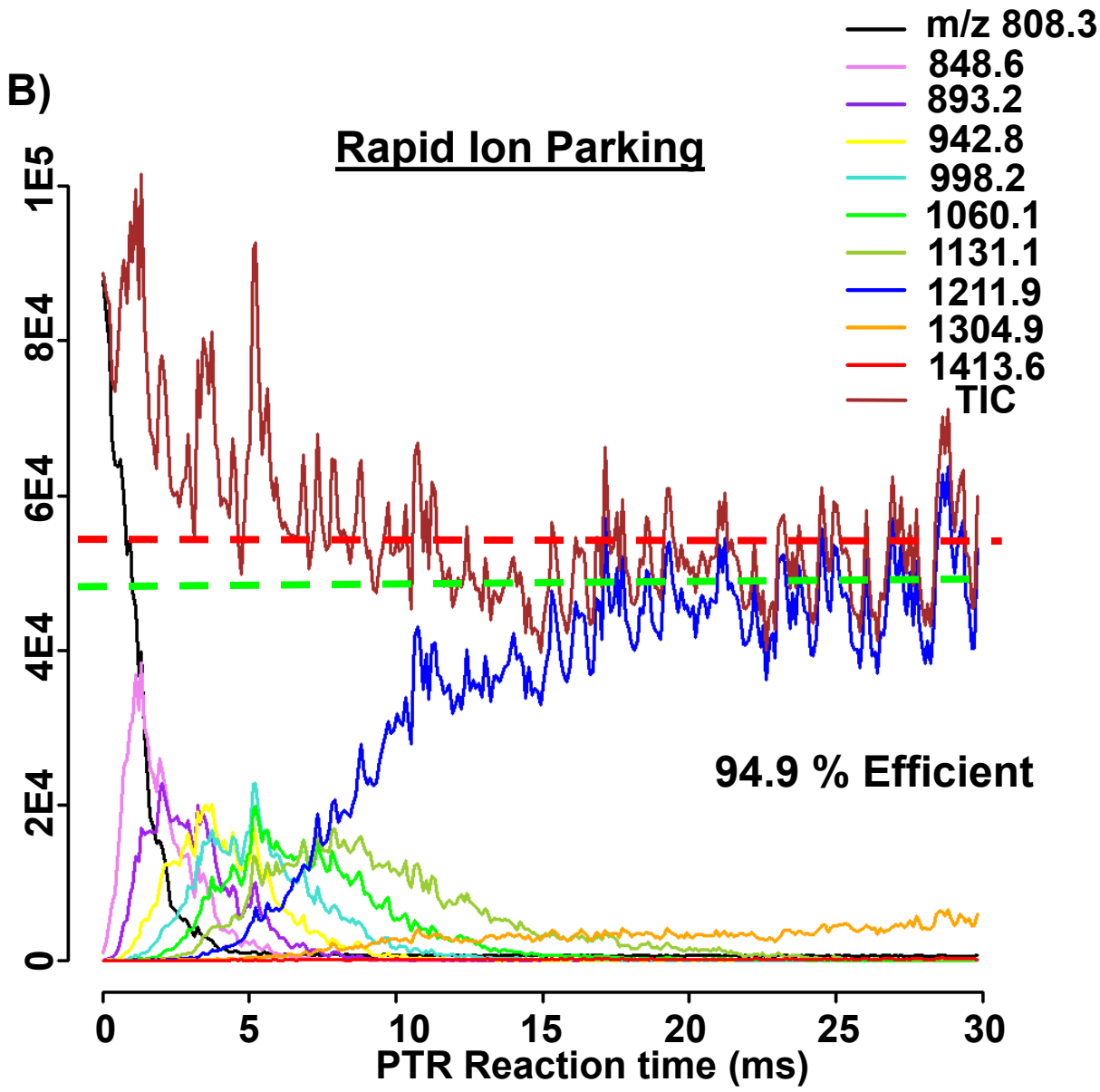

B)
Rapid Ion Parking
m/z 808.3
848.6
893.2
942.8
998.2
1060.1
1131.1
1211.9
1304.9
1413.6
TIC
94.9 % Efficient
0
2E4
4E4
6E4
8E4
1E5
0
5
10
15
20
25
30
PTR Reaction time (ms)

Figure S8

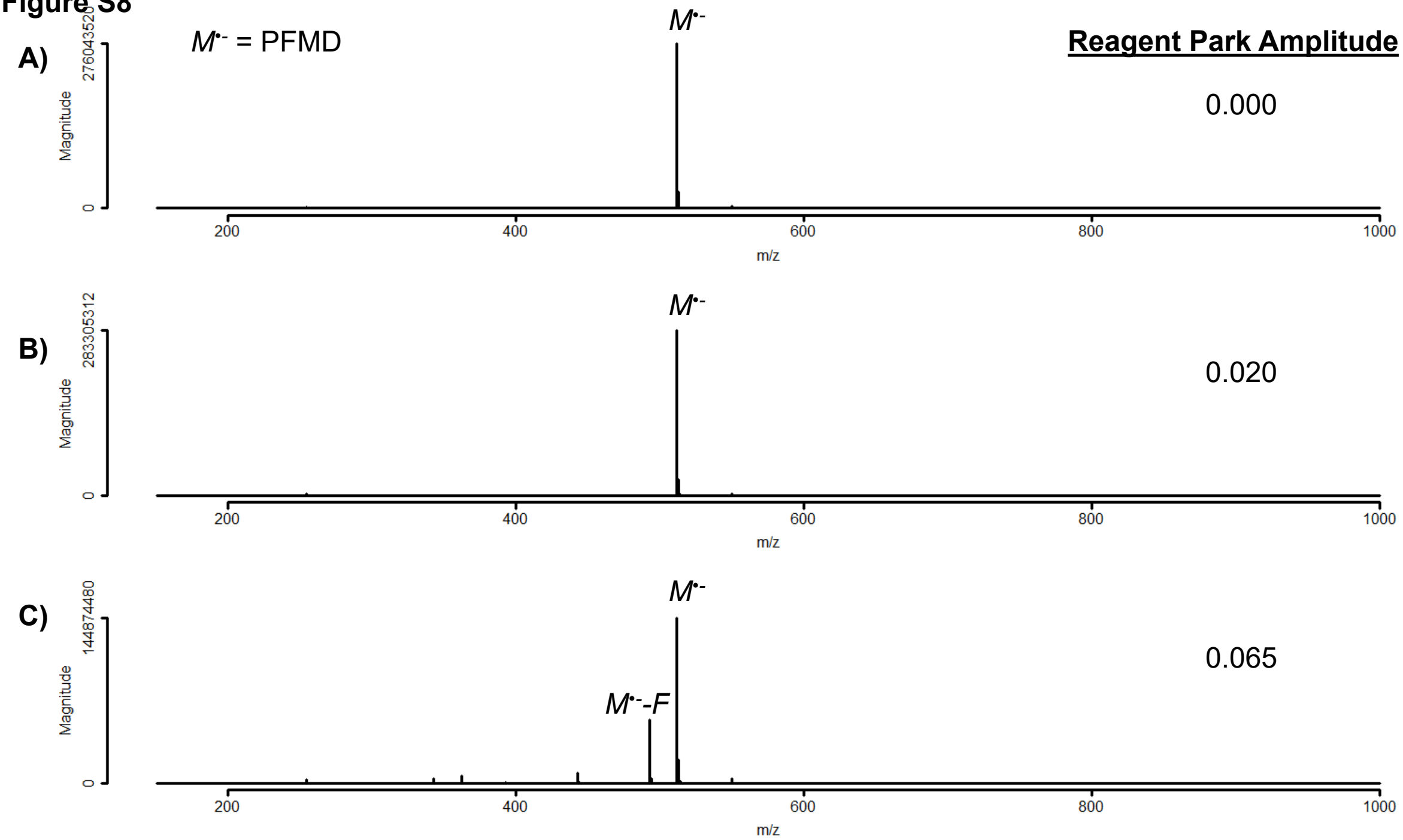

A)
$M^{\bullet-}$ = PFMD
Reagent Park Amplitude
0.000
$M^{\bullet-}$
Magnitude
276043520
0
200
400
600
800
1000
m/z
B)
0.020
$M^{\bullet-}$
Magnitude
283305312
0
200
400
600
800
1000
m/z
C)
0.065
$M^{\bullet-}$
$M^{\bullet-}$-F
Magnitude
144874480
0
200
400
600
800
1000
m/z

Figure S9

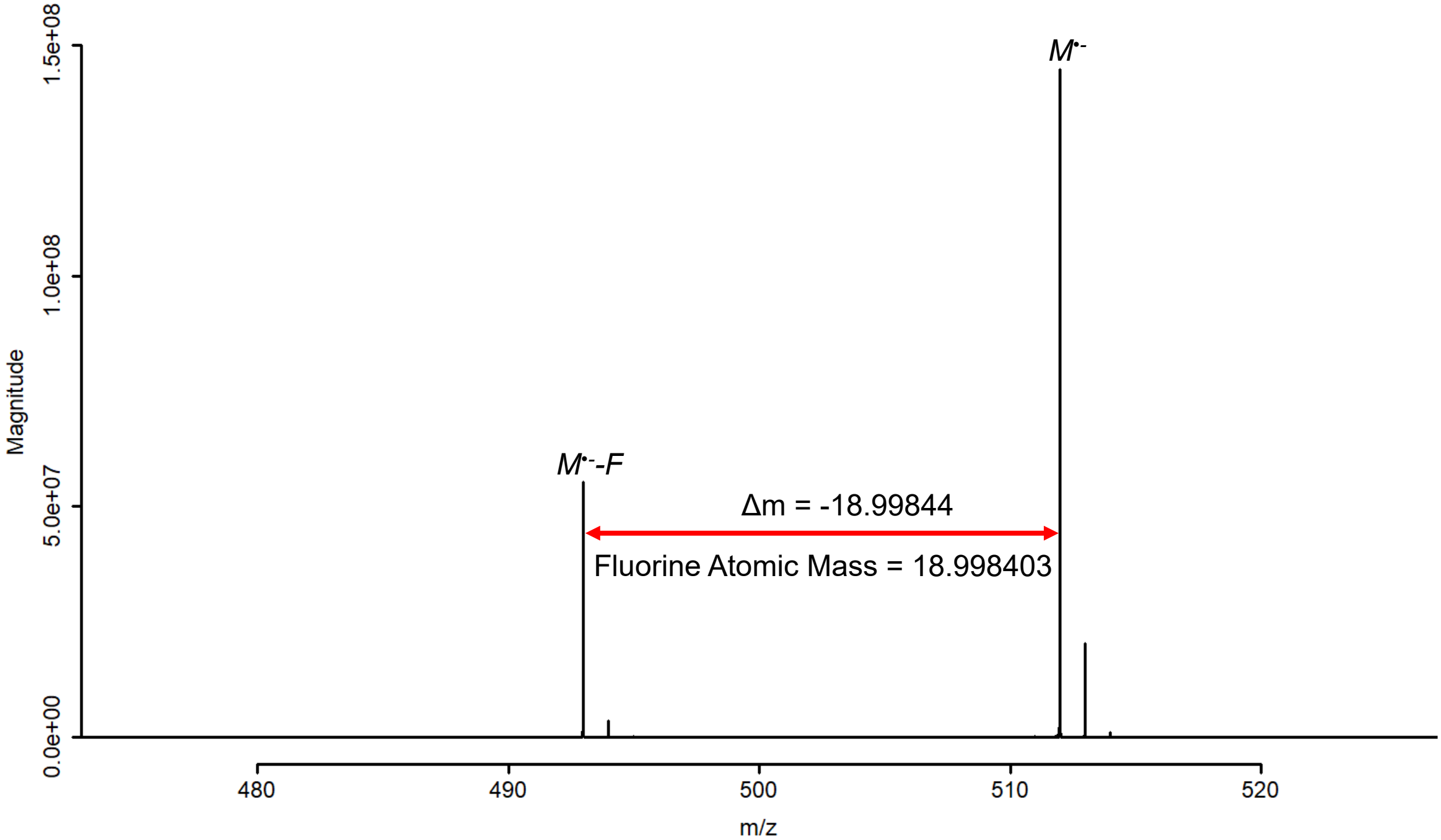

1.5e+08
1.0e+08
5.0e+07
0.0e+00
Magnitude
M•-
M•--F
Δm = -18.99844
Fluorine Atomic Mass = 18.998403
480
490
500
510
520
m/z

Figure S10

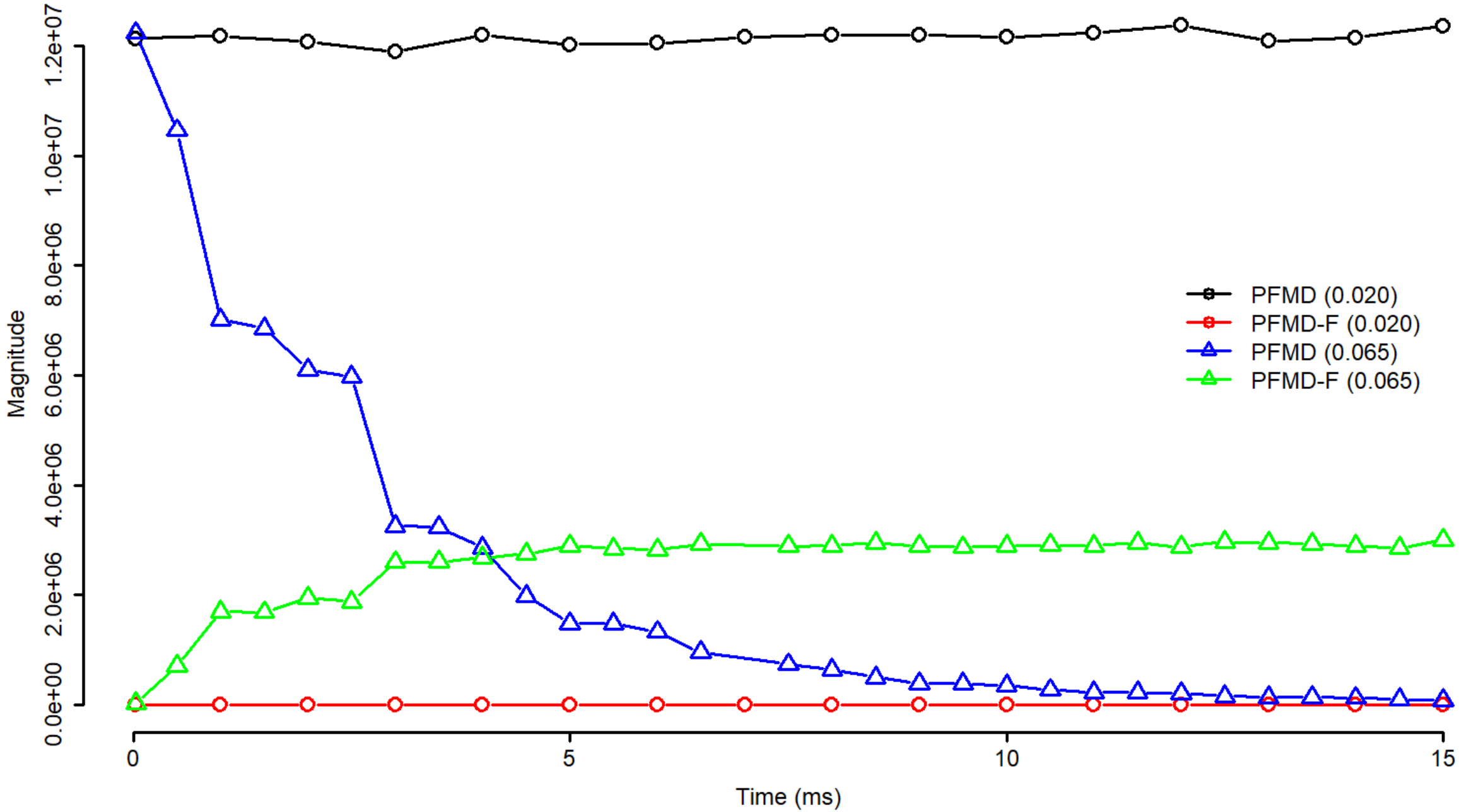

Magnitude
0.0e+00
2.0e+06
4.0e+06
6.0e+06
8.0e+06
1.0e+07
1.2e+07
0
5
10
15
Time (ms)
PFMD (0.020)
PFMD-F (0.020)
PFMD (0.065)
PFMD-F (0.065)

Figure S11

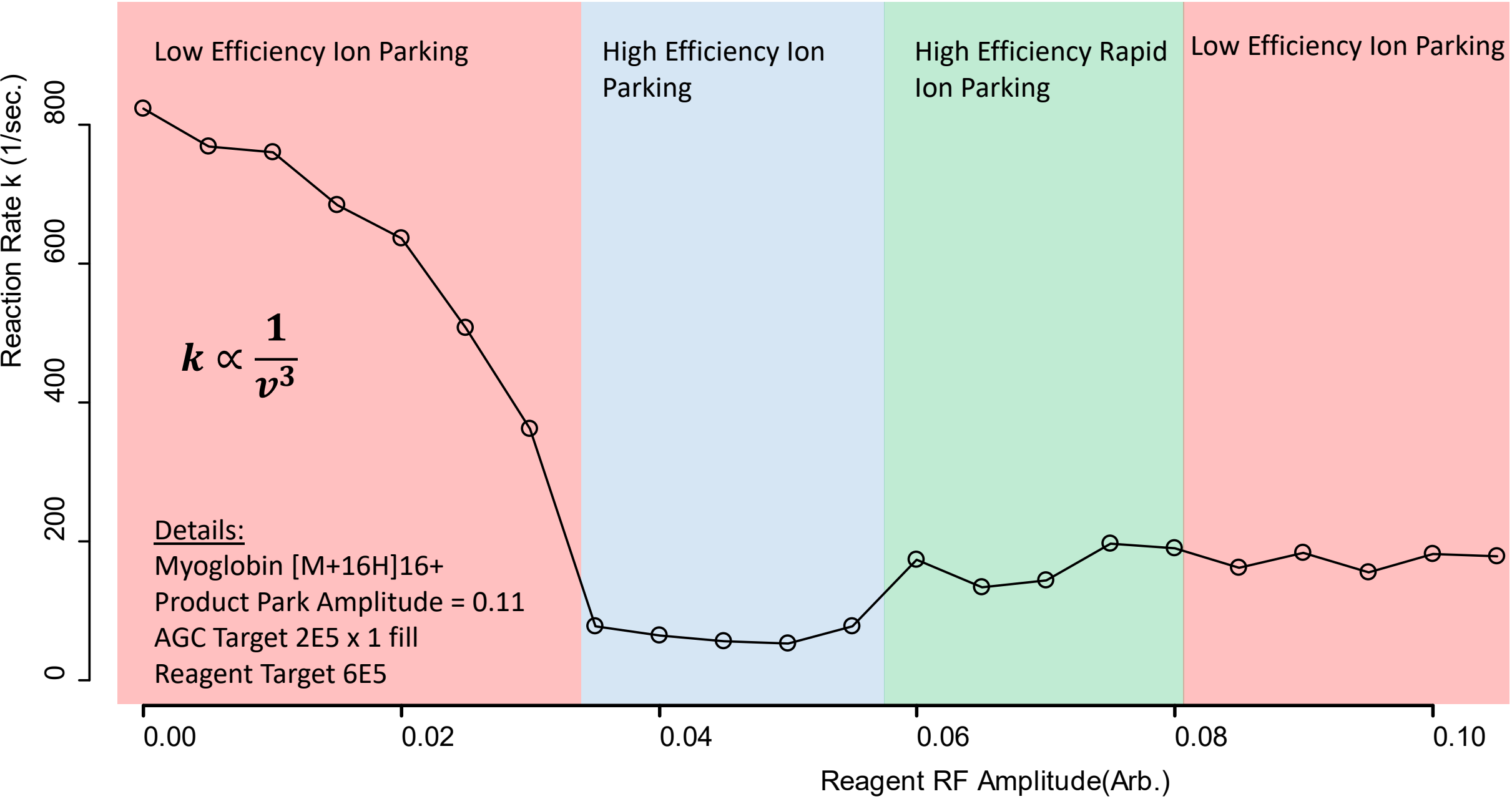
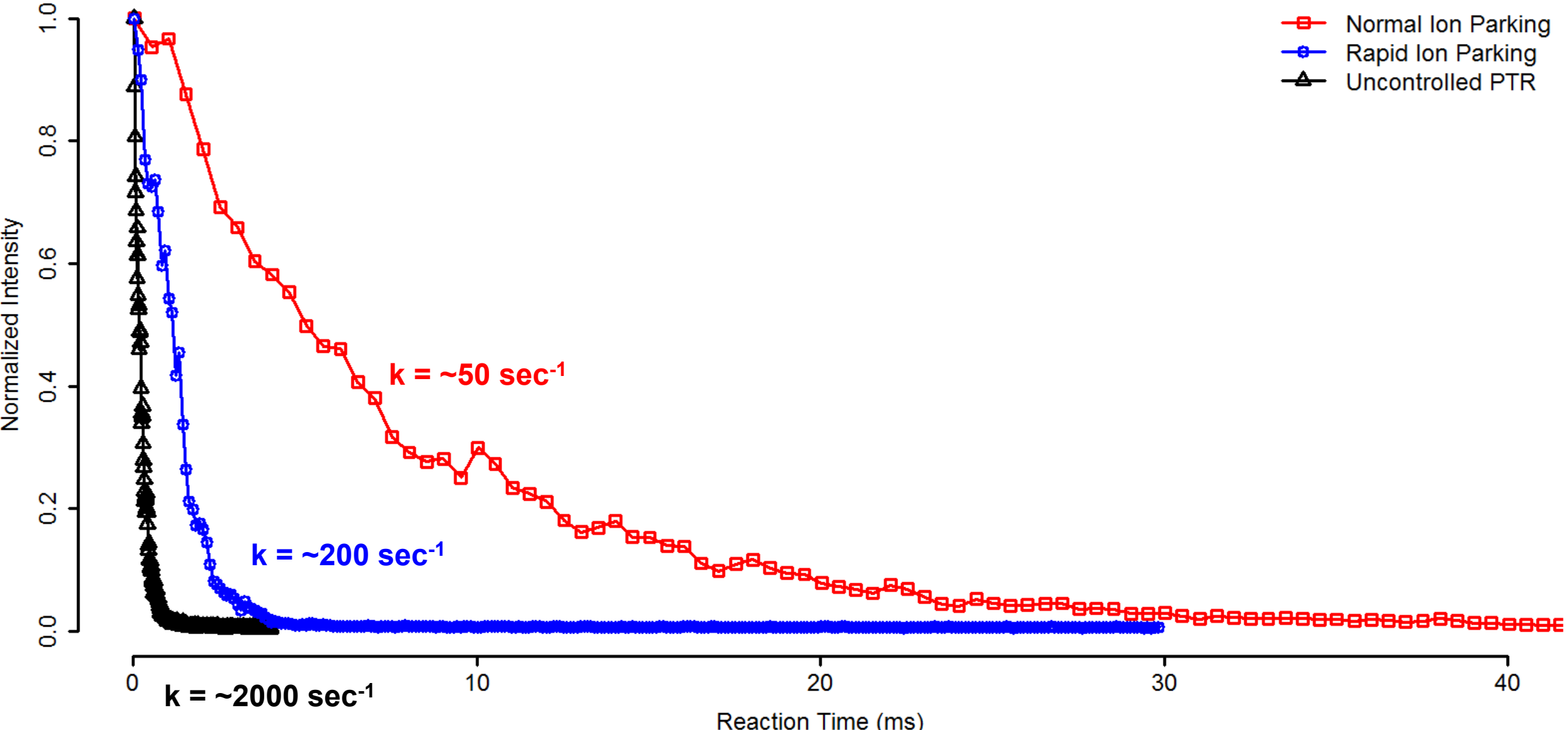

Low Efficiency Ion Parking
High Efficiency Ion Parking
High Efficiency Rapid Ion Parking
Low Efficiency Ion Parking
Reaction Rate k (1/sec.)
0
200
400
600
800
$k \propto \frac{1}{v^3}$
Details:
Myoglobin [M+16H]16+
Product Park Amplitude = 0.11
AGC Target 2E5 x 1 fill
Reagent Target 6E5
0.00
0.02
0.04
0.06
0.08
0.10
Reagent RF Amplitude(Arb.)

Figure S12

C)


Normal Ion Parking
Rapid Ion Parking
Uncontrolled PTR
k = ~50 sec-1
k = ~200 sec-1
k = ~2000 sec-1
Normalized Intensity
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
Reaction Time (ms)