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Supporting Information

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for

Processing of N-Terminal Unnatural Amino Acids in Recombinant Human Interferon-β in *Escherichia coli*

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David A. Tirrell, and Kenneth Grabstein

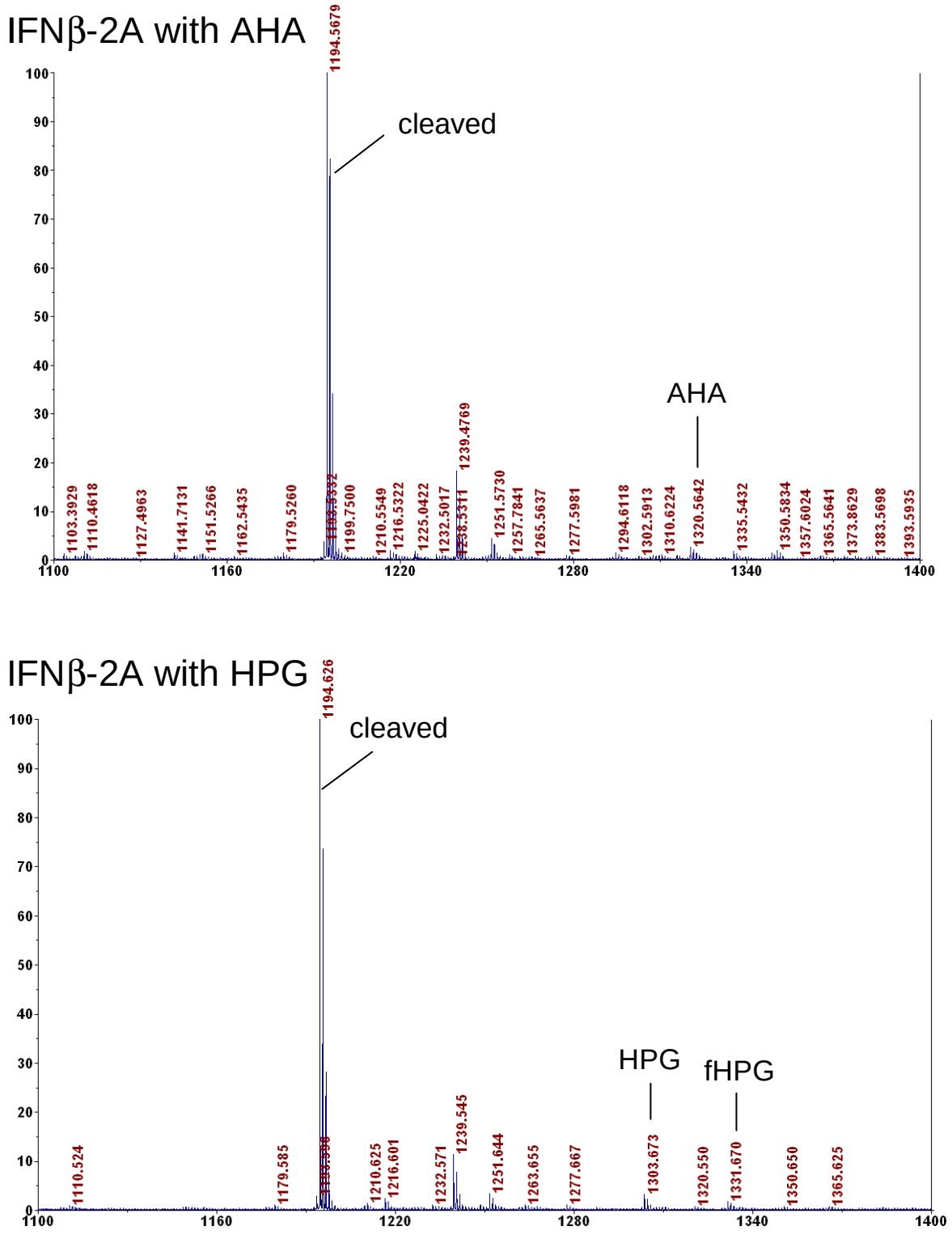
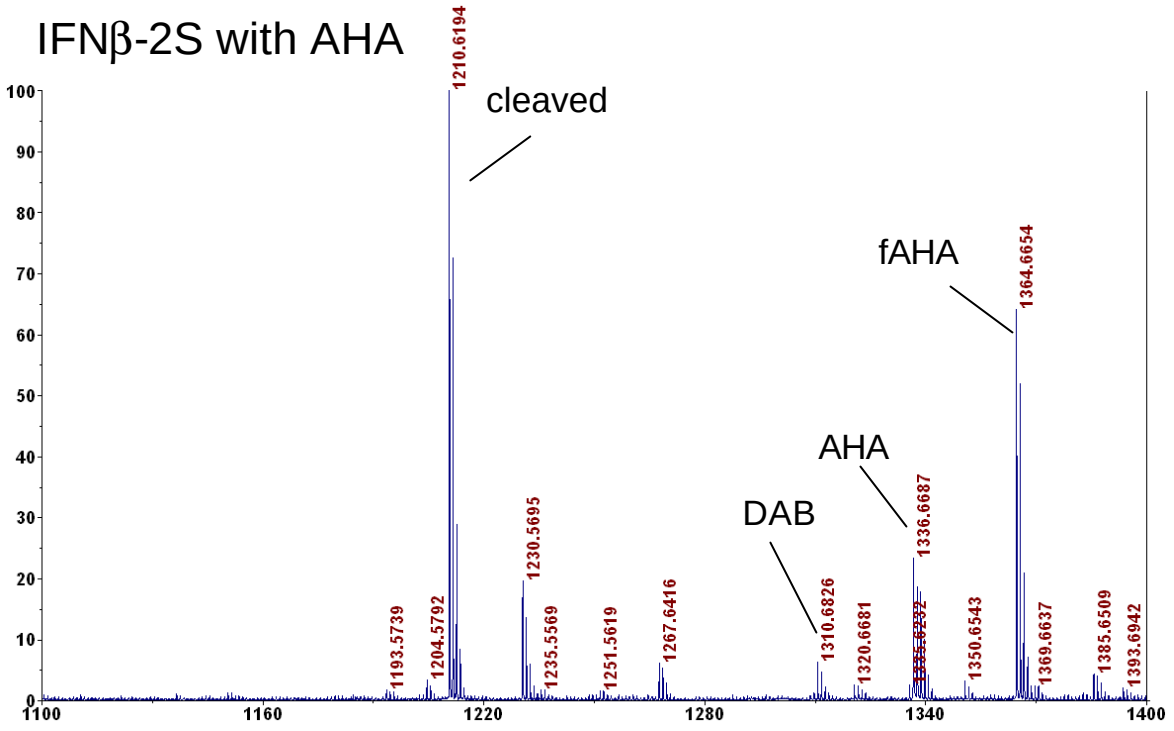
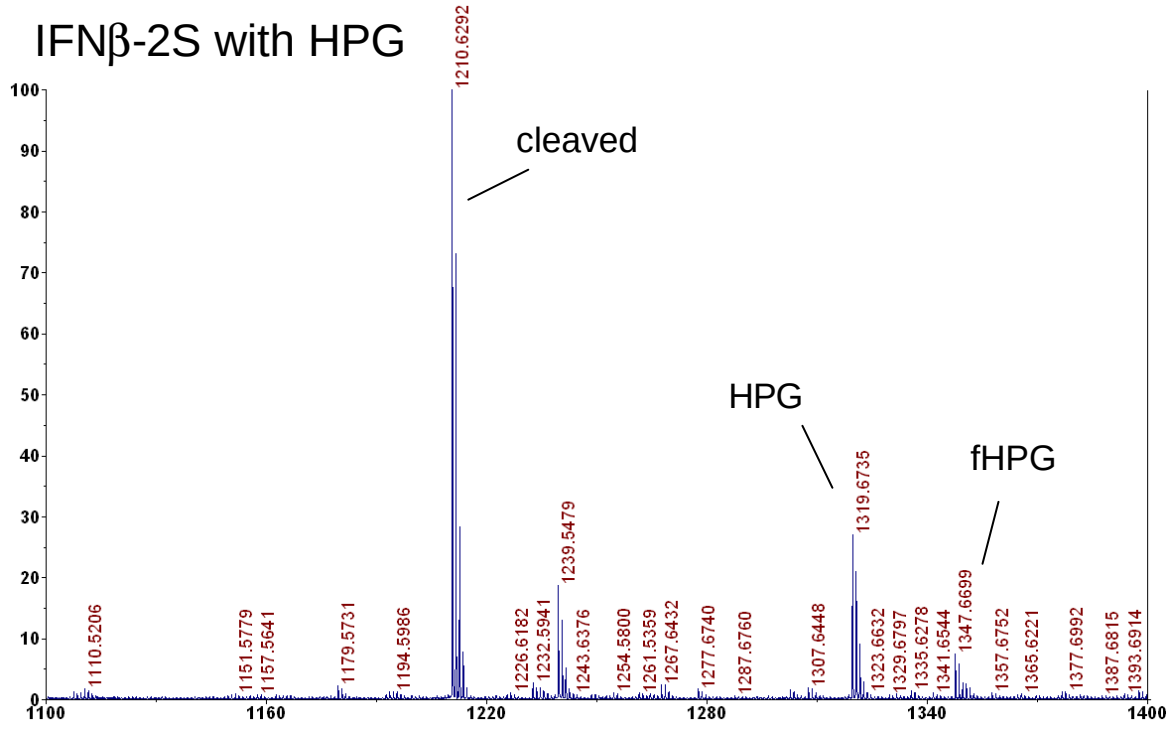
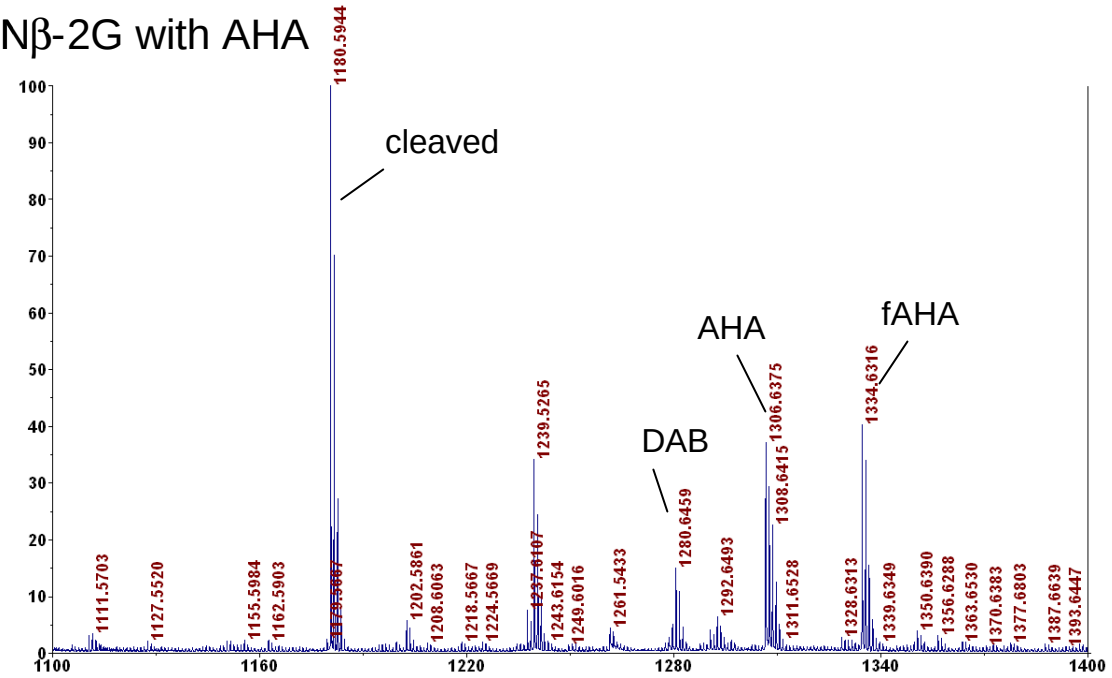


Figure S1. MALDI mass spectra of the tryptic peptides for all IFN? β protein variants expressed with AHA or HPG (Table 1). The N-terminal peptide variants are labeled. The signal at 1239.5 is assigned to an internal IFNβ peptide.

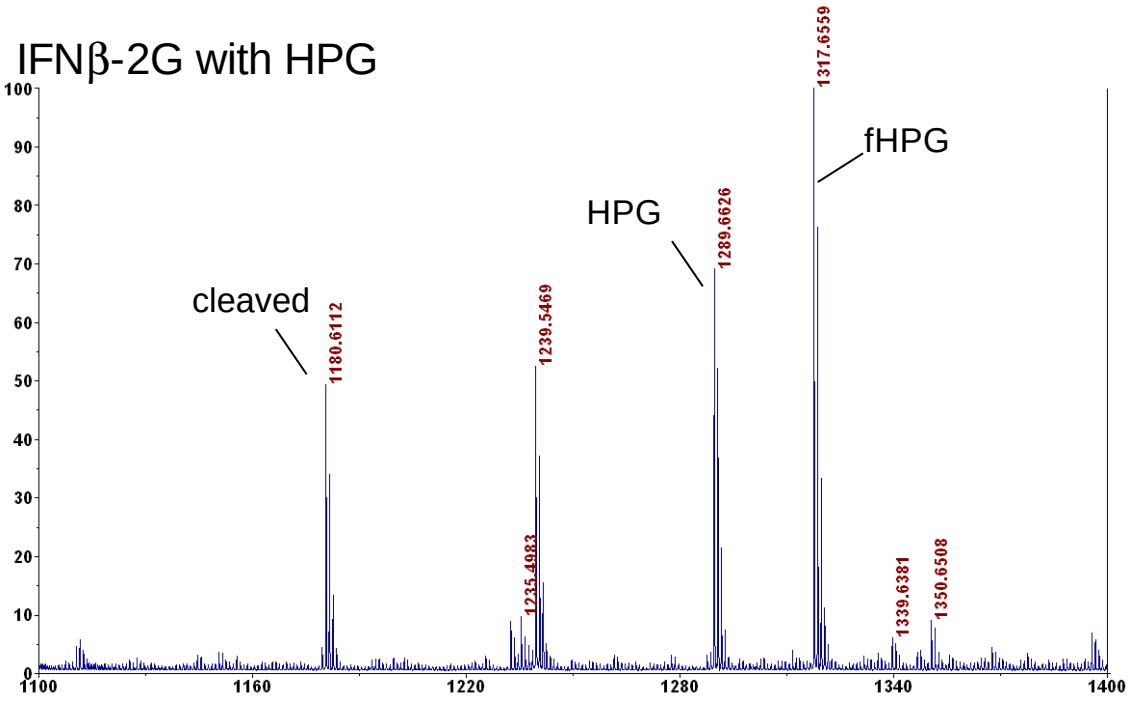

IFNβ-2S with AHA
1210.6194
cleaved
fAHA
1364.6654
AHA
1336.6687
DAB
1310.6826
1230.5695
1193.5739
1204.5792
1235.5569
1251.5619
1267.6416
1320.6681
1350.6543
1369.6637
1385.6509
1393.6942
100
90
80
70
60
50
40
30
20
10
0
1100
1160
1220
1280
1340
1400

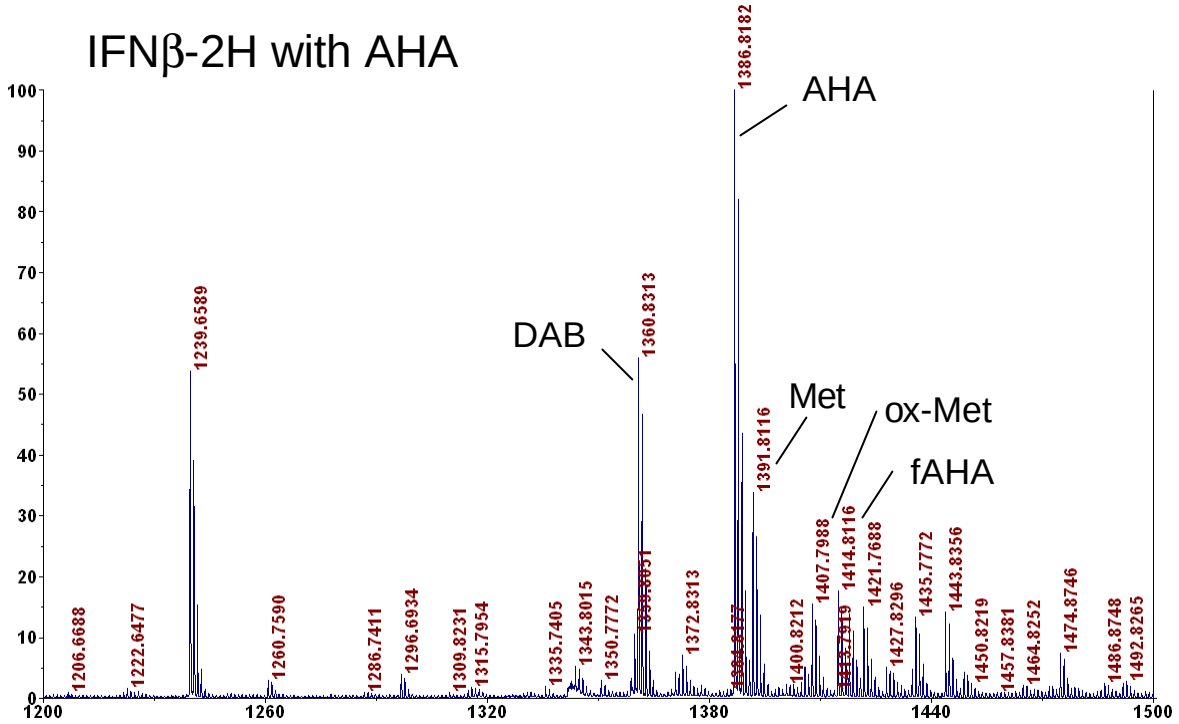

IFNβ-2S with HPG
1210.6292
cleaved
HPG
1319.6735
fHPG
1239.5479
1110.5206
1151.5779
1157.5641
1179.5731
1194.5986
1226.6182
1232.5941
1243.6376
1254.5800
1261.5359
1267.6432
1277.6740
1287.6760
1307.6448
1323.6632
1329.6797
1335.6278
1341.6544
1347.6699
1357.6752
1365.6221
1377.6992
1387.6815
1393.6914
100
90
80
70
60
50
40
30
20
10
0
1100
1160
1220
1280
1340
1400

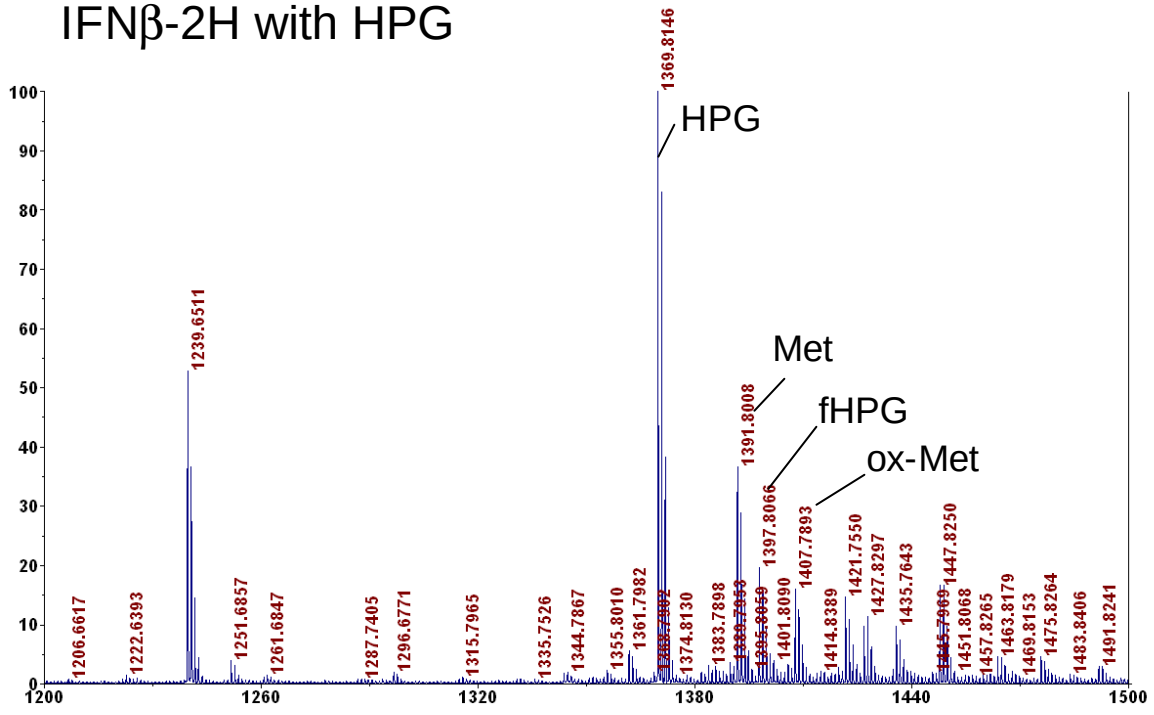
IFNβ-2G with AHA



IFNβ-2G with HPG




IFNβ-2H with AHA
AHA
DAB
Met
ox-Met
fAHA
1386.8182
1239.6589
1360.8313
1391.8116
1407.7988
1414.8116
1421.7688
1206.6688
1222.6477
1260.7590
1286.7411
1296.6934
1309.8231
1315.7954
1335.7405
1343.8015
1350.7772
1372.8313
1400.8212
1427.8296
1435.7772
1443.8356
1450.8219
1457.8381
1464.8252
1474.8746
1486.8748
1492.8265
100
90
80
70
60
50
40
30
20
10
0
1200
1260
1320
1380
1440
1500


IFNβ-2H with HPG
HPG
Met
fHPG
ox-Met
1369.8146
1239.6511
1391.8008
1397.8066
1407.7893
1206.6617
1222.6393
1251.6857
1261.6847
1287.7405
1296.6771
1315.7965
1335.7526
1344.7867
1355.8010
1361.7982
1374.8130
1383.7898
1401.8090
1414.8389
1421.7550
1427.8297
1435.7643
1447.8250
1451.8068
1457.8265
1463.8179
1469.8153
1475.8264
1483.8406
1491.8241
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40
30
20
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0
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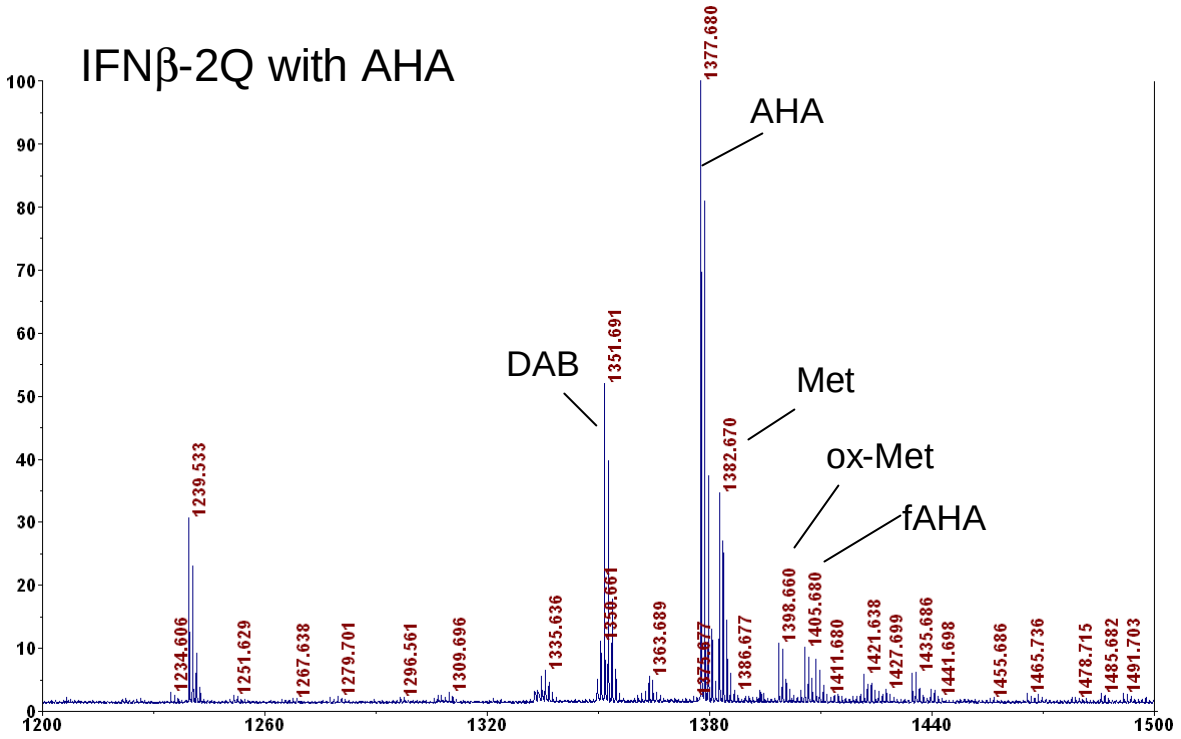
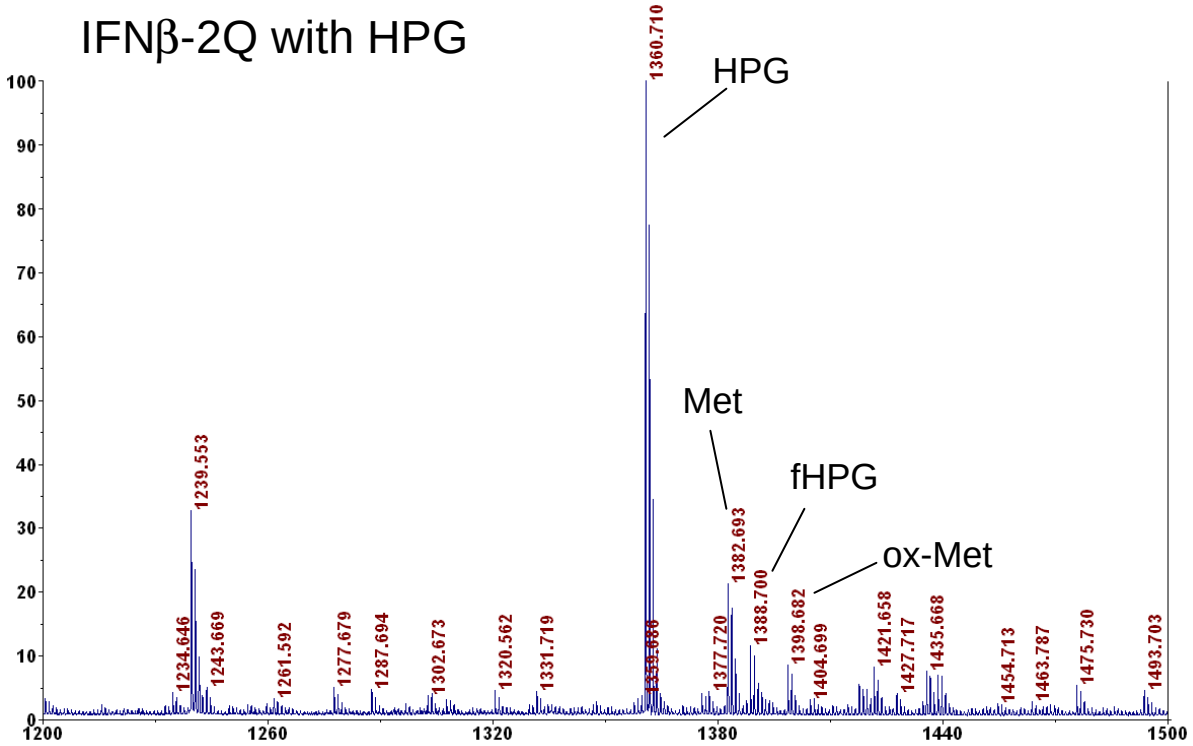
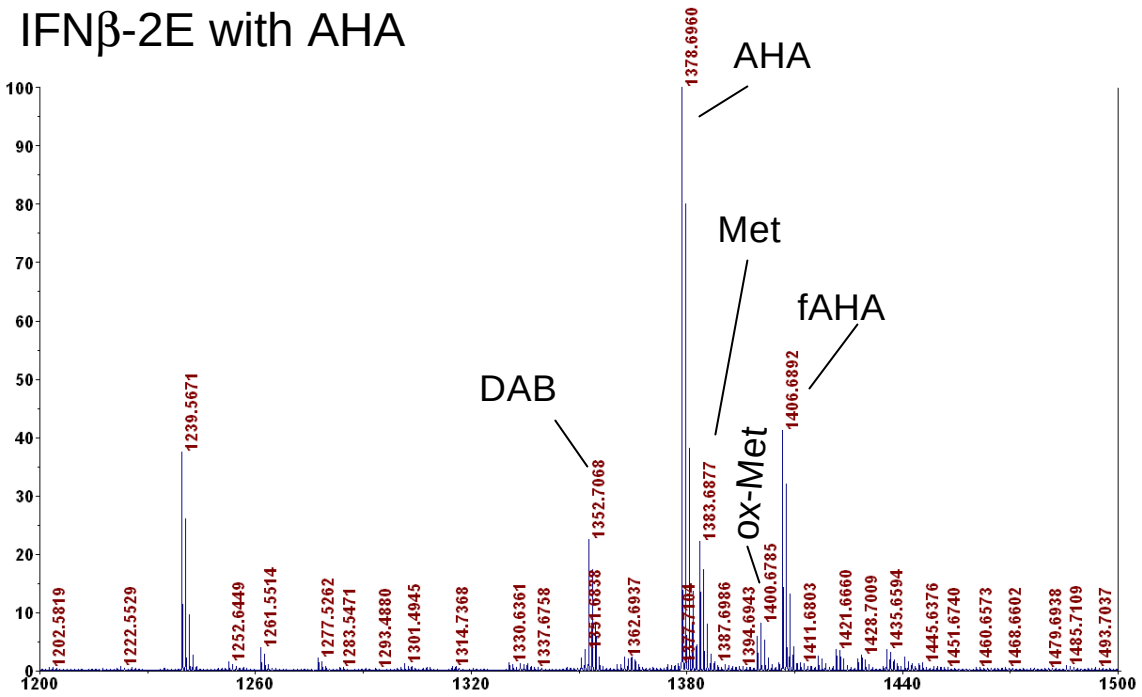
1260
1320
1380
1440
1500


IFNβ-2Q with AHA
AHA
DAB
Met
ox-Met
fAHA
1377.680
1351.691
1382.670
1239.533
1234.606
1251.629
1267.638
1279.701
1296.561
1309.696
1335.636
1350.661
1363.689
1375.677
1386.677
1398.660
1405.680
1411.680
1421.638
1427.699
1435.686
1441.698
1455.686
1465.736
1478.715
1485.682
1491.703
100
90
80
70
60
50
40
30
20
10
0
1200
1260
1320
1380
1440
1500


IFNβ-2Q with HPG
HPG
Met
fHPG
ox-Met
1360.710
1382.693
1239.553
1234.646
1243.669
1261.592
1277.679
1287.694
1302.673
1320.562
1331.719
1377.720
1388.700
1398.682
1404.699
1421.658
1427.717
1435.668
1454.713
1463.787
1475.730
1493.703
100
90
80
70
60
50
40
30
20
10
0
1200
1260
1320
1380
1440
1500


IFNβ-2E with AHA
AHA
Met
fAHA
DAB
ox-Met
1378.6960
1239.5671
1352.7068
1406.6892
1383.6877
1400.6785
1202.5819
1222.5529
1252.6449
1261.5514
1277.5262
1283.5471
1293.4880
1301.4945
1314.7368
1330.6361
1337.6758
1362.6937
1387.6986
1394.6943
1411.6803
1421.6660
1428.7009
1435.6594
1445.6376
1451.6740
1460.6573
1468.6602
1479.6938
1485.7109
1493.7037
100
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80
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60
50
40
30
20
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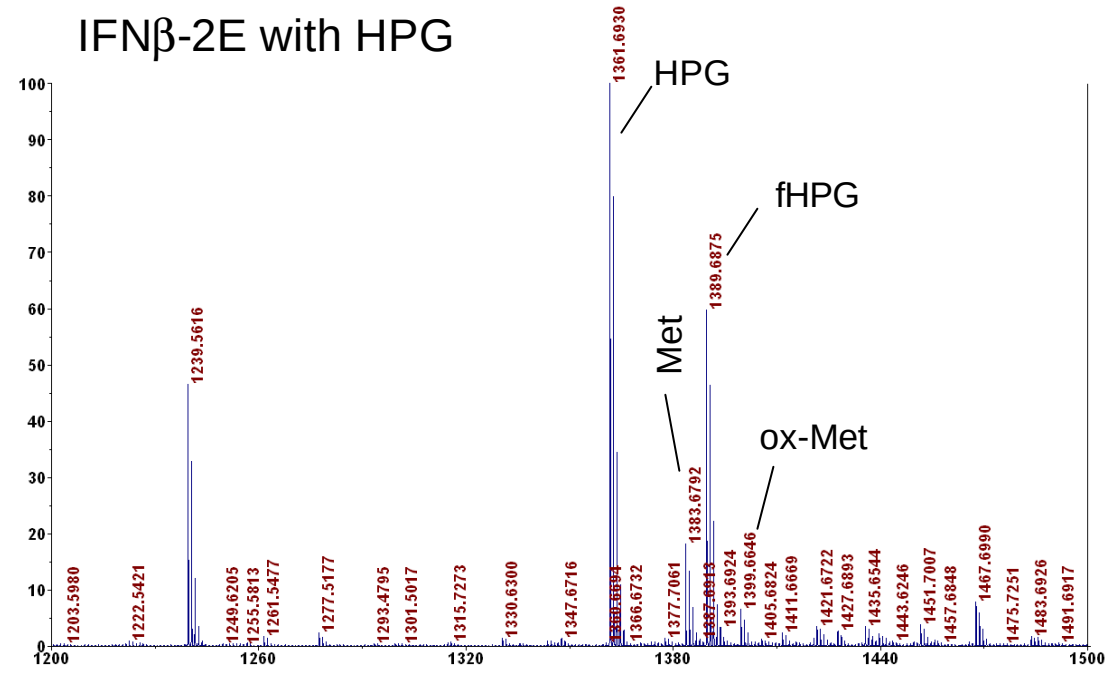
0
1200
1260
1320
1380
1440
1500


IFNβ-2E with HPG
HPG
fHPG
Met
ox-Met
1361.6930
1239.5616
1389.6875
1383.6792
1203.5980
1222.5421
1249.6205
1255.5813
1261.5477
1277.5177
1293.4795
1301.5017
1315.7273
1330.6300
1347.6716
1366.6732
1377.7061
1393.6924
1399.6646
1405.6824
1411.6669
1421.6722
1427.6893
1435.6544
1443.6246
1451.7007
1457.6848
1467.6990
1475.7251
1483.6926
1491.6917
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1320
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1440
1500

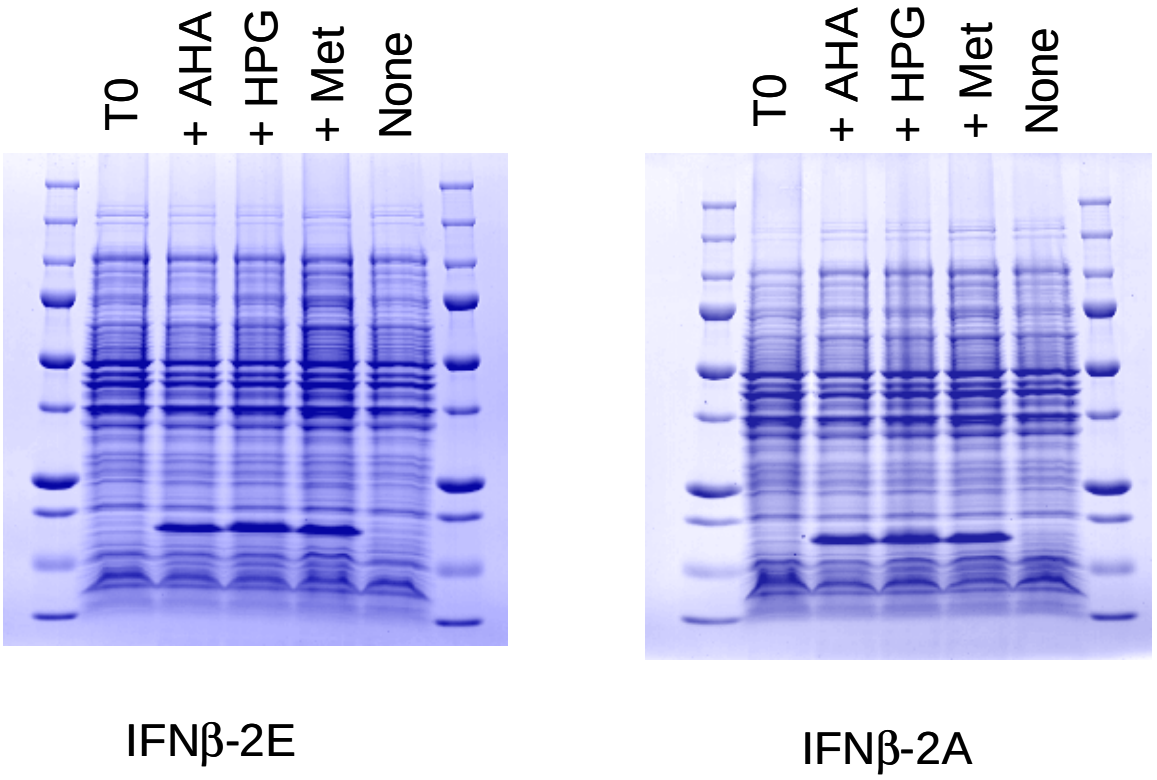

Figure S2. SDS-PAGE (4-20%) of recombinant IFNβ-2E and IFNβ-2A expression profiles. *E. coli* cell lysates prior to induction (T0), and 2 h post IPTG induction in Met free medium supplemented with AHA, HPG, Met, or no Met or analog, were analyzed and protein bands were visualized by Coomassie Blue staining. The protein markers in lane 1 and 7 are 250, 150, 100, 75, 50, 37, 25, 20,15 and 10 kDa.