

BMB/Bi/Ch 173 – Winter 2017

Problem Set 8.2: Mass Spectrometry– Assigned 3-2-17. Due 3-7-17 by 10:30am

TA: Wen Zhou (201 Kerckoff, office hour: Fri 3/3 5-6pm, Mon 3/6 5-6pm or by appointment

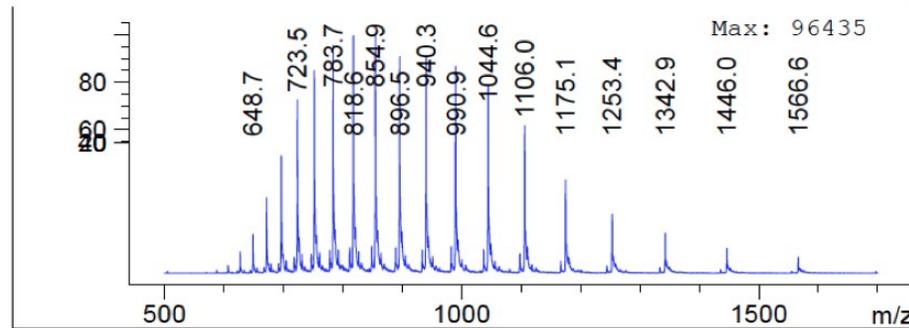
100 points total

1. (10 points) Ionization Basics:

What is the difference between hard and soft ionization techniques? Describe a scenario in which one would use a soft ionization technique rather than a hard one.

2. (20 points) Mass Spec of Intact Proteins:

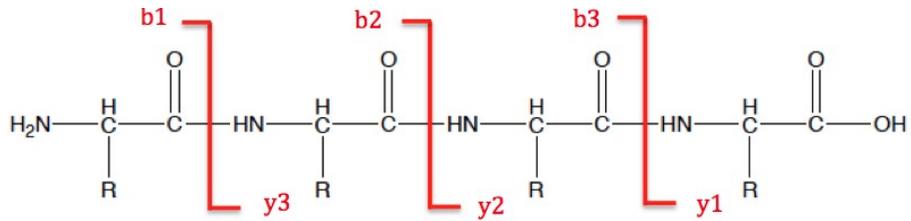
- a. (8 Points) Below is the mass spectrum of an intact protein. Why are there multiple peaks in the spectrum if the protein has not been digested? What ionization technique was used?



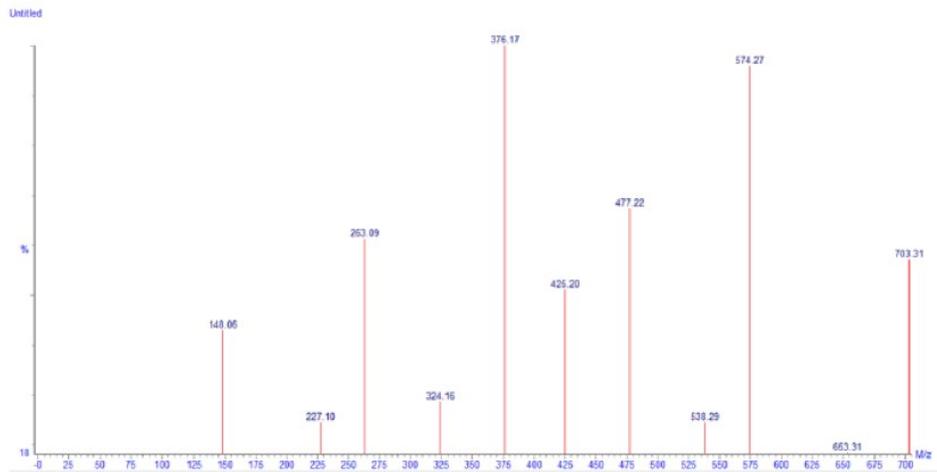
- b. (12 points) Determine the mass of the protein. Show your work. (Hint: use the formulas: $(M + nH)/n = (m/z)_n$ and $(M + (n-1)H)/(n-1) = (m/z)_{(n-1)}$)

3. (35 points) Peptide Analysis:

- a. (20 points) When peptides are fragmented, they are broken into pieces at their amide bonds. This process forms a series of b-ions (with C-terminal acylium ions) and y-ions (with N-terminal ammonium ions), as shown:



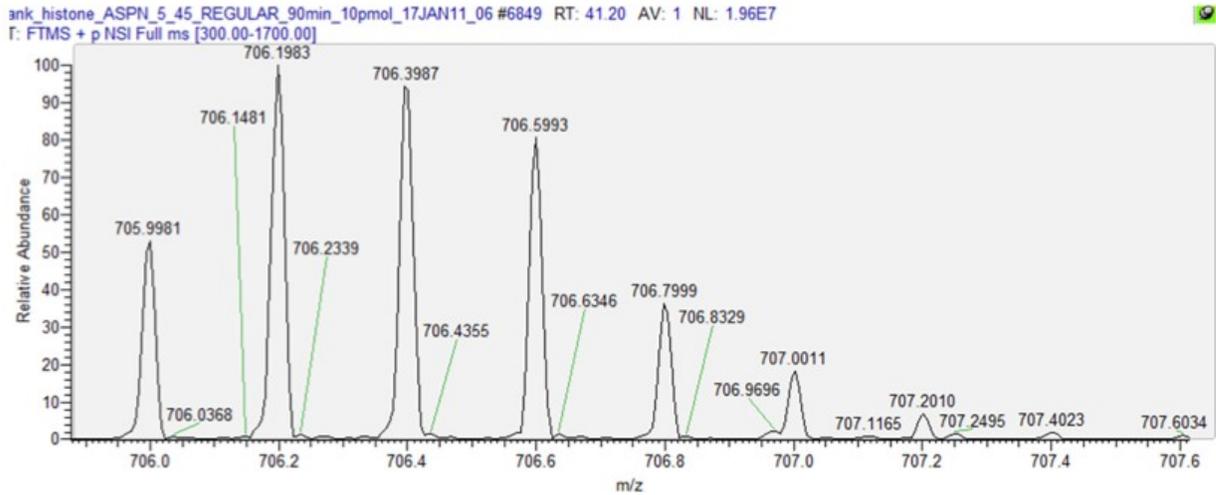
Shown below are the b- and y-ion series of a fragmented peptide ion with a molecular weight of 799.360 Da. The singly charged precursor was measured at 800.3638 Da. Using the spectrum, determine the amino acid sequence of the peptide. On the spectrum, please label which peaks correspond to which b- and y- ions (b_1 , b_2 , etc.) and give the sequence of each ion. The residual weights of the amino acids are listed in tables below (you only need to consider the monoisotopic masses). Be sure to show your work. For clarity, the peaks in the spectrum have the following m/z values: 148.06, 227.10, 263.09, 324.16, 376.17, 425.20, 477.22, 538.29, 574.27, 653.31, and 703.31



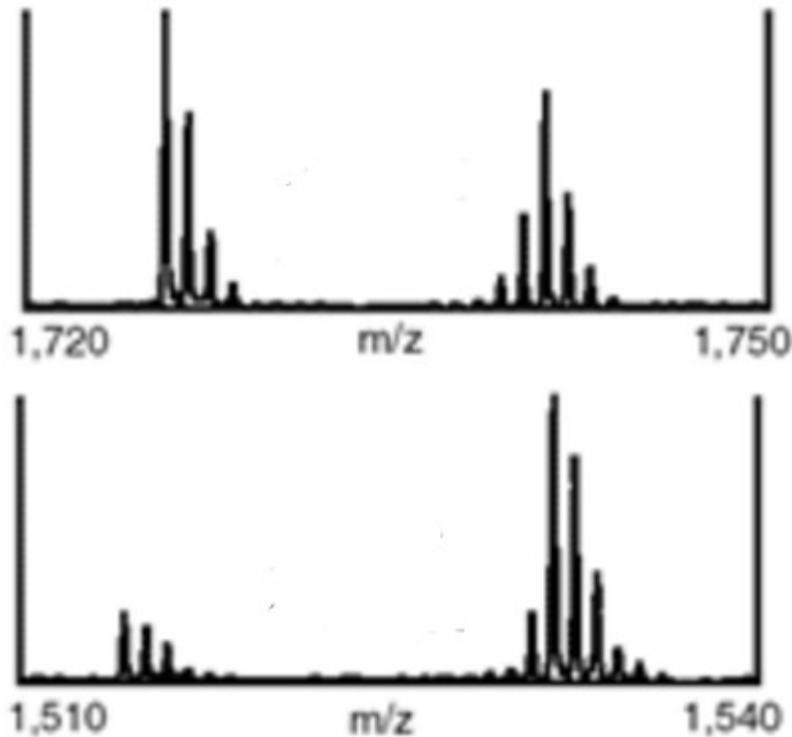
Residual molecular weights of amino acids

Name (Symbols)	Residue Composition	Residue Structure	Average Mass	Monoisotopic Mass	Sidechain Mass	Immonium Ion Mass (related i)
Alanine (Ala or A)	C₃H₅NO		71.0788	71.03711	15	44
Arginine (Arg or R)	C₆H₁₂N₄O		156.1876	156.10111	100	129 (112, 129, 146, 163, 180, 197, 214, 231, 248, 265, 282, 299, 316, 333, 350, 367, 384, 401, 418, 435, 452, 469, 486, 503, 520, 537, 554, 571, 588, 605, 622, 639, 656, 673, 690, 707, 724, 741, 758, 775, 792, 809, 826, 843, 860, 877, 894, 911, 928, 945, 962, 979, 996, 1013, 1030, 1047, 1064, 1081, 1098, 1115, 1132, 1149, 1166, 1183, 1200, 1217, 1234, 1251, 1268, 1285, 1302, 1319, 1336, 1353, 1370, 1387, 1404, 1421, 1438, 1455, 1472, 1489, 1506, 1523, 1540, 1557, 1574, 1591, 1608, 1625, 1642, 1659, 1676, 1693, 1710, 1727, 1744, 1761, 1778, 1795, 1812, 1829, 1846, 1863, 1880, 1897, 1914, 1931, 1948, 1965, 1982, 1999, 2016, 2033, 2050, 2067, 2084, 2101, 2118, 2135, 2152, 2169, 2186, 2203, 2220, 2237, 2254, 2271, 2288, 2305, 2322, 2339, 2356, 2373, 2390, 2407, 2424, 2441, 2458, 2475, 2492, 2509, 2526, 2543, 2560, 2577, 2594, 2611, 2628, 2645, 2662, 2679, 2696, 2713, 2730, 2747, 2764, 2781, 2798, 2815, 2832, 2849, 2866, 2883, 2900, 2917, 2934, 2951, 2968, 2985, 3002, 3019, 3036, 3053, 3070, 3087, 3104, 3121, 3138, 3155, 3172, 3189, 3206, 3223, 3240, 3257, 3274, 3291, 3308, 3325, 3342, 3359, 3376, 3393, 3410, 3427, 3444, 3461, 3478, 3495, 3512, 3529, 3546, 3563, 3580, 3597, 3614, 3631, 3648, 3665, 3682, 3699, 3716, 3733, 3750, 3767, 3784, 3801, 3818, 3835, 3852, 3869, 3886, 3903, 3920, 3937, 3954, 3971, 3988, 4005, 4022, 4039, 4056, 4073, 4090, 4107, 4124, 4141, 4158, 4175, 4192, 4209, 4226, 4243, 4260, 4277, 4294, 4311, 4328, 4345, 4362, 4379, 4396, 4413, 4430, 4447, 4464, 4481, 4498, 4515, 4532, 4549, 4566, 4583, 4600, 4617, 4634, 4651, 4668, 4685, 4702, 4719, 4736, 4753, 4770, 4787, 4804, 4821, 4838, 4855, 4872, 4889, 4906, 4923, 4940, 4957, 4974, 4991, 5008, 5025, 5042, 5059, 5076, 5093, 5110, 5127, 5144, 5161, 5178, 5195, 5212, 5229, 5246, 5263, 5280, 5297, 5314, 5331, 5348, 5365, 5382, 5399, 5416, 5433, 5450, 5467, 5484, 5501, 5518, 5535, 5552, 5569, 5586, 5603, 5620, 5637, 5654, 5671, 5688, 5705, 5722, 5739, 5756, 5773, 5790, 5807, 5824, 5841, 5858, 5875, 5892, 5909, 5926, 5943, 5960, 5977, 5994, 6011, 6028, 6045, 6062, 6079, 6096, 6113, 6130, 6147, 6164, 6181, 6198, 6215, 6232, 6249, 6266, 6283, 6300, 6317, 6334, 6351, 6368, 6385, 6402, 6419, 6436, 6453, 6470, 6487, 6504, 6521, 6538, 6555, 6572, 6589, 6606, 6623, 6640, 6657, 6674, 6691, 6708, 6725, 6742, 6759, 6776, 6793, 6810, 6827, 6844, 6861, 6878, 6895, 6912, 6929, 6946, 6963, 6980, 6997, 7014, 7031, 7048, 7065, 7082, 7099, 7116, 7133, 7150, 7167, 7184, 7201, 7218, 7235, 7252, 7269, 7286, 7303, 7320, 7337, 7354, 7371, 7388, 7405, 7422, 7439, 7456, 7473, 7490, 7507, 7524, 7541, 7558, 7575, 7592, 7609, 7626, 7643, 7660, 7677, 7694, 7711, 7728, 7745, 7762, 7779, 7796, 7813, 7830, 7847, 7864, 7881, 7898, 7915, 7932, 7949, 7966, 7983, 8000, 8017, 8034, 8051, 8068, 8085, 8102, 8119, 8136, 8153, 8170, 8187, 8204, 8221, 8238, 8255, 8272, 8289, 8306, 8323, 8340, 8357, 8374, 8391, 8408, 8425, 8442, 8459, 8476, 8493, 8510, 8527, 8544, 8561, 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11213, 11230, 11247, 11264, 11281, 11298, 11315, 11332, 11349, 11366, 11383, 11400, 11417, 11434, 11451, 11468, 11485, 11502, 11519, 11536, 11553, 11570, 11587, 11604, 11621, 11638, 11655, 11672, 11689, 11706, 11723, 11740, 11757, 11774, 11791, 11808, 11825, 11842, 11859, 11876, 11893, 11910, 11927, 11944, 11961, 11978, 11995, 12012, 12029, 12046, 12063, 12080, 12097, 12114, 12131, 12148, 12165, 12182, 12199, 12216, 12233, 12250, 12267, 12284, 12301, 12318, 12335, 12352, 12369, 12386, 12403, 12420, 12437, 12454, 12471, 12488, 12505, 12522, 12539, 12556, 12573, 12590, 12607, 12624, 12641, 12658, 12675, 12692, 12709, 12726, 12743, 12760, 12777, 12794, 12811, 12828, 12845, 12862, 12879, 12896, 12913, 12930, 12947, 12964, 12981, 12998, 13015, 13032, 13049, 13066, 13083, 13100, 13117, 13134, 13151, 13168, 13185, 13202, 13219, 13236, 13253, 13270, 13287, 13304, 13321, 13338, 13355, 13372, 13389, 13406, 13423, 13440, 13457, 13474, 13491, 13508, 13525, 13542, 13559, 13576, 13593, 13610, 13627, 13644, 13661, 13678, 13695, 13712, 13729, 13746, 13763, 13780, 13797, 13814, 13831, 13848, 13865, 13882, 13899, 13916, 13933, 13950, 13967, 13984, 14001, 14018, 14035, 14052, 14069, 14086, 14103, 14120, 14137, 14154, 14171, 14188, 14205, 14222, 14239, 14256, 14273, 14290, 14307, 14324, 14341, 14358, 14375, 14392, 14409, 14426, 14443, 14460, 14477, 14494, 14511, 14528, 14545, 14562, 14579, 14596, 14613, 14630, 14647, 14664, 14681, 14698, 14715, 14732, 14749, 14766, 14783, 14800, 14817, 14834, 14851, 14868, 14885, 14902, 14919, 14936, 14953, 14970, 14987, 15004, 15021, 15038, 15055, 15072, 15089, 15106, 15123, 15140, 15157, 15174, 15191, 15208, 15225, 15242, 15259, 15276, 15293, 15310, 15327, 15344, 15361, 15378, 15395, 15412, 15429, 15446, 15463, 15480, 15497, 15514, 15531, 15548, 15565, 15582, 15599, 15616, 15633, 15650, 15667, 15684, 15701, 15718, 15735, 15752, 15769, 15786, 15803, 15820, 15837, 15854, 15871, 15888, 15905, 15922, 15939, 15956, 15973, 15990, 16007, 16024, 16041, 16058, 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28233, 28250, 28267, 28284, 28301, 28318, 28335, 28352, 28369, 28386, 28403, 28420, 28437, 28454, 28471, 28488, 28505, 28522, 28539, 28556, 28573, 28590, 28607, 28624, 28641, 28658, 28675, 28692, 28709, 28726, 28743, 28760, 28777, 28794, 28811, 28828, 28845, 28862, 28879, 28896, 28913, 28930, 28947, 28964, 28981, 29000, 29017, 29034, 29051, 29068, 29085, 29102, 29119, 29136, 29153, 29170, 29187, 29204, 29221, 29238, 29255, 29272, 29289, 29306, 29323, 29340, 29357, 29374, 29391, 29408, 29425, 29442, 29459, 29476, 29493, 29510, 29527, 29544, 29561, 29578, 29595, 29612, 29629, 29646, 29663, 29680, 29697, 29714, 29731, 29748, 29765, 29782, 29799, 29816, 29833, 29850, 29867, 29884, 29901, 29918, 29935, 29952, 29969, 29986, 30003, 30020, 30037, 30054, 30071, 30088, 30105, 30122, 30139, 30156, 30173, 30190, 30207, 30224, 30241, 30258, 30275, 30292, 30309, 30326, 30343, 30360, 30377, 30394, 30411, 30428, 30445, 30462, 30479, 30496, 30513, 30530,

b. (15 points) Shown below is part of the MS spectrum of an AspN digested peptide from a thymus calf histone. Note: the small peaks labeled with green lines represent low abundance fragments and can be ignored. What is the charge state of this peptide fragment? What is the molecular weight of this peptide? For your calculation, use 1.00784 as the mass of a proton. Show your work.



- ii. (10 points) You label the WT worms with “heavy” and the KO worms with “light.” Below are spectra of peptides from two different proteins. Why are there multiple peaks for the “light” and “heavy” samples? Why are the patterns of intensities of these multiple peaks different between “light” and “heavy”?



- iii. (5 points) Estimate the relative abundances of light versus heavy peptides in each of the two spectra (ie what is the fold-change). Is each protein up-regulated, down-regulated, or unaffected by the KO of WoRM1?
- c. (8 points) You get some interesting hits from your initial experiment, and you want to know which of the proteins physically interact with WoRM1. You decide to do a co-immunoprecipitation of WoRM1 and use mass spec to identify its binding partners. There are no available antibodies to WoRM1,

so you decide to introduce an exogenous epitope-tagged version of WoRM1. You plan on co-IPing WoRM1 using an antibody to this tag and identifying the bound proteins by mass spec in the same way you identified the proteins in the SILAC experiment (except without isotopic labeling). Explain two potential problems that could arise from using this method rather than co-IPing endogenous untagged WoRM1.