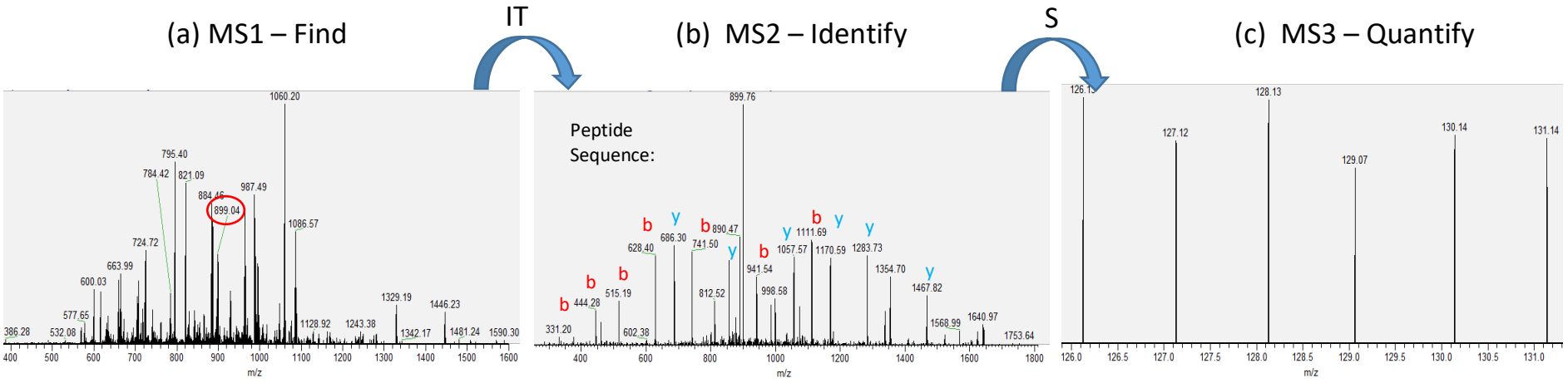

#1	b+	b2+	Seq.	y+	y2+	#2
1	331.21789	166.11258	T-TMT6plex			15
2	444.30195	222.65461	I	1466.85771	733.93249	14
3	515.33906	258.17317	A	1353.77364	677.39046	13
4	628.42313	314.71520	I	1282.73653	641.87190	12
5	741.50719	371.25723	I	1169.65246	585.32987	11
6	812.54431	406.77579	A	1056.56840	528.78784	10
7	941.58690	471.29709	E	985.53129	493.26928	9
8	998.60836	499.80782	G	856.48869	428.74798	8
9	1111.69243	556.34985	I	799.46723	400.23725	7
10	1208.74519	604.87623	P	686.38317	343.69522	6
11	1337.78778	669.39753	E	589.33040	295.16884	5
12	1408.82490	704.91609	A	460.28781	230.64754	4
13	1521.90896	761.45812	L	389.25069	195.12899	3
14	1622.95664	811.98196	T	276.16663	138.58695	2
15			R	175.11895	88.06311	1

Discovery-Based Quantitative Proteomics Workflow. Individual biological protein extracts are labelled with unique Tandem Mass Tags (TMTs) before being pooled for LC-MS analysis. (a) During LC-MS, TMT-labelled peptides are delivered to the mass spectrometer via ionization, and target peptides are selected during a primary scan (MS1). (b) The target peptide is subjected to collision-induced dissociation (CID) which preferentially cleaves peptide bonds, thereby revealing sequence information (MS2). The table inset demonstrates how b- and y-ion assignment can determine peptide sequence. (c) Finally, the target peptide is subjected to high-energy collision dissociation which cleaves the TMT reporter ion and yields relative quantitation across all samples in the pooled extract (MS3). This process is repeated on a novel target peptide in a non-biased selection until all peptides have been analyzed (see *Methods* for more detailed information).