Supplemental Table S1. Identified target peptides at 1% FDR.

	Mascot	SEQUEST	MS Amanda
HCD HeLa Replicate 1	8,924	9,417	10,841
HCD HeLa Replicate 2	10,025	10,909	12,101
HCD HeLa Replicate 3	10,119	10,530	11,773
HCD PepLib Phospho	26,495	22,976	27,414
HCD PepLib No Phospho	31,835	34,861	35,418
ETD PepLib Phospho	6,196	-	6,890
ETD PepLib No Phospho	6,368	-	8,018
CID HeLa Replicate 1	5,288	5,372	5,442
CID HeLa Replicate 2	5,342	5,356	5,642
CID HeLa Replicate 3	5,576	5,560	5,746

Supplemental Table S2. Identified target PSMs at 1% FDR.

	Mascot	SEQUEST	MS Amanda
HCD HeLa Replicate 1	12,386	12,858	15,091
HCD HeLa Replicate 2	13,761	14,628	16,305
HCD HeLa Replicate 3	13,537	14,223	15,984
HCD Histone	620	778	969
HCD PepLib Phospho	72,771	62,031	75,605
HCD PepLib No Phospho	103,999	112,491	118,491
ETD PepLib Phospho	12,016	-	12,979
ETD PepLib No Phospho	13,489	-	16,400
CID HeLa Replicate 1	6,895	6,961	7,046
CID HeLa Replicate 2	7,093	7,077	7,409
CID HeLa Replicate 3	7,091	7,152	7,373

Supplemental Table S3. Performance comparison of MS Amanda and Morpheus on HCD HeLa data set[30] at 1% FDR. Search settings and modifications were as described above: 7 ppm precursor mass tolerance, 0.03 Da fragment mass tolerance, Oxidation (M) as variable modification, Carbamidomethyl (C) as fixed modification, and trypsin as enzyme allowing up to two missed cleavages.

	Morpheus	MS Amanda
HCD HeLa Replicate 1	12,060	15,091
HCD HeLa Replicate 2	12,019	16,305
HCD HeLa Replicate 3	11,068	15,984
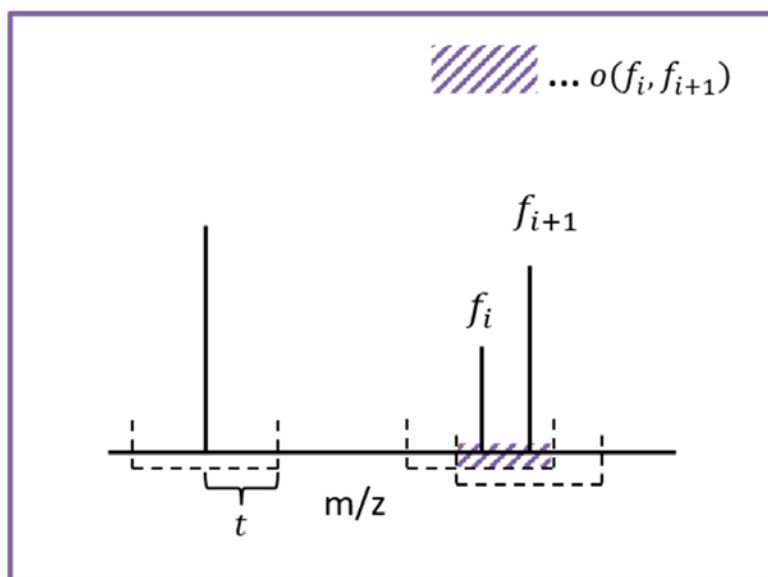
Supplemental Figure S1. Schematic view of overlap calculation for fragments ions given mass tolerance t (see Formula 4). Each peak covers an m/z range of 2*t, leading to potential overlaps of covered range for nearby peaks. The probability to match a peak by chance is given by the fraction of covered range and total m/z range. For calculation of the covered range overlapping areas (marked areas in Figure) are subtracted from the sum of all peak ranges.