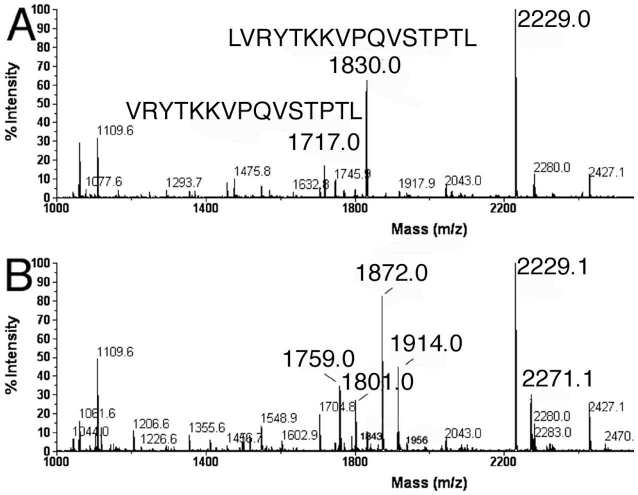

Figure S1. MALDI-TOF mass spectra of pepsin digested human albumin (15 µM) before (A) and after (B) treatment with 0.5 mM p-nitrophenyl acetate for 6 h. The 1717 amu peak for VRYTKKVPQVSTPTL in panel A has acquired +42, +84, and +126 amu masses at 1759, 1801, and 1843 amu in panel B. The 1830 amu peak for LVRYTKKVPQVSTPTL in panel A has acquired +42, +84, and +126 amu masses in panel B at 1872, 1914, and 1956 amu.

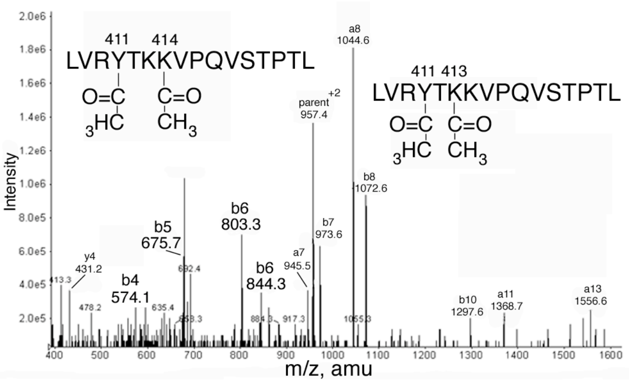

Figure S2. MS/MS spectrum to identify acetylated sites in LVRYTKKVPQVSTPTL after 6 h reaction of 1 mg/ml albumin with 0.5 mM p-nitrophenyl acetate. The doubly charged parent ion at 957.4 m/z, representing the singly charged 1914 amu ion, carries two acetyl groups. The acetyl groups are distributed between Y411/K414 and Y411/K413. The dominant ion is acetylated on Y411 and K414, while a less abundant ion is acetylated on Y411 and K413.

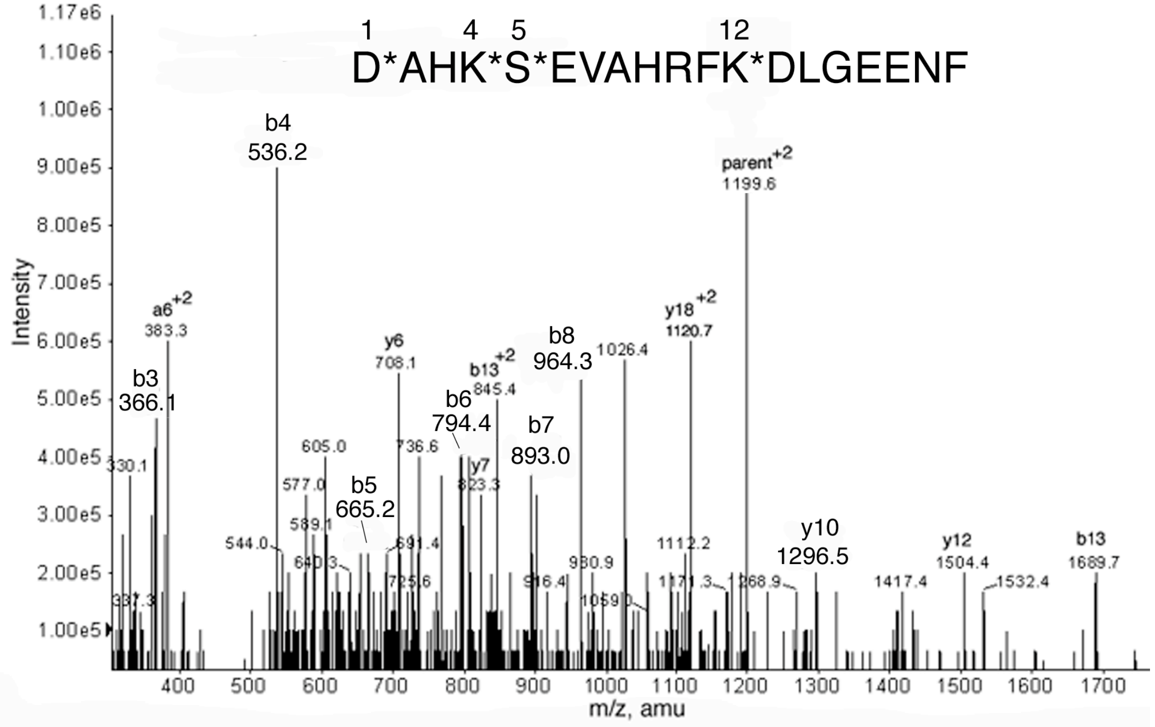

Figure S3. MS/MS spectrum to identify 4 acetylation sites on DAHKSEVAHRFKDLGEENF. Human albumin (15 µM) was incubated with 10 mM p-nitrophenyl acetate for 48 h at pH 8.0, before digestion with pepsin. The 4 acetylated residues in ion 2397 are D1, K4, S5, and K12.

Table S1. Mass spectrometry identification of residues in human albumin (accession number gi: 3212456) acetylated by 10 mM p-nitrophenyl acetate.

Start - End	Mr	Sequence	Acetylated	Protease	missed cleavages
1 - 19	2396.06	DAHKSEVAHRFKDLGEENF	D1 K4 S5 K12	pepsin	4
1 - 20	2482.19	DAHKSEVAHRFKDLGEENFK	D1 K4 K12	trypsin	3
7 - 17	1341.67	VAHRFKDLGEE	K12	Glu-C	1
11 - 20	1267.61	FKDLGEENFK	K12	trypsin	1
18 - 45	3417.74	NFKALVLIAFAQYLQQCPFEDHVKLVNE	K20 K41	Glu-C	1
18 - 45	3375.73	NFKALVLIAFAQYLQQCPFEDHVKLVNE	K41	Glu-C	1
42 - 64	2670.18	LVNEVTEFAKTCVADESAENCDK	K51	trypsin	1
49 - 57	1081.47	FAKTCVADE	K51	Glu-C	0
49 - 60	1368.59	FAKTCVADESAE	K51	Glu-C	1
61 - 82	2661.29	NCDKSLHTLFGDKLCTVATLRE	K64 K73	Glu-C	0
61 - 82	2619.28	NCDKSLHTLFGDKLCTVATLRE	K73	Glu-C	0
61 - 82	2661.29	NCDKSLHTLFGDKLCTVATLRE	S65 K73	Glu-C	0
65 - 81	2015.05	SLHTLFGDKLCTVATLR	S65 K73	trypsin	1
65 - 81	2015.05	SLHTLFGDKLCTVATLR	T68 K73	trypsin	1
65 - 81	1973.04	SLHTLFGDKLCTVATLR	T76	trypsin	1
65 - 81	1973.04	SLHTLFGDKLCTVATLR	K73	trypsin	1
74 - 98	3171.36	LCTVATLRETYGEM$_{OX}$ADCCAKQEPER	T76 T79 T83 K93	trypsin	2
74 - 98	3197.38	LCTVATLRETYGEMADCCAKQEPER	T76 T79 T83 Y84 K93	trypsin	2
82 - 98	2114.83	ETYGEMADCCAKQEPER	K93	trypsin	1
87 -100	1778.70	MADCCAKQEPERNE	K93	Glu-C	2
101 - 119	2389.20	CFLQHKDDNPNLPRLVRPE	K106	Glu-C	0
133 - 141	1245.66	TFLKKYLYE	K137	Glu-C	0
133 - 141	1287.68	TFLKKYLYE	K136 K137	Glu-C	0

137 - 144	1096.59	KYLYEIAR	K137	trypsin	1
146 - 162	2315.18	HPYFYAPELLFFAKRYK	K159 Y161 K162	trypsin	2
154 - 167	1745.95	LLFFAKRYKAAFTE	K159	Glu-C	0
154 - 167	1745.95	LLFFAKRYKAAFTE	Y161	Glu-C	0
154 - 167	1787.96	LLFFAKRYKAAFTE	Y161 K162	Glu-C	0
154 - 167	1787.96	LLFFAKRYKAAFTE	K159 K162	Glu-C	0
161 - 174	1703.73	YKAAFTECCQAADK	K162	trypsin	1
161 - 181	2499.16	YKAAFTECCQAADKAACLLPK	K162 K174	trypsin	2
161 - 186	3167.51	YKAAFTECCQAADKAACLLPKLDELR	K162 K174 K181	trypsin	3
163 - 181	2165.99	AAFTECCQAADKAACLLPK	K174	trypsin	1
168 - 184	2003.91	CCQAADKAACLLPKLDE	K174	Glu-C	0
168 - 184	2045.92	CCQAADKAACLLPKLDE	K174 K181	Glu-C	0
168 - 188	2517.17	CCQAADKAACLLPKLDELRDE	K174	Glu-C	1
168 - 188	2559.18	CCQAADKAACLLPKLDELRDE	K174 K181	Glu-C	1
175 - 186	1439.78	AACLLPKLDELR	K181	trypsin	1
175 - 195	2313.20	AACLLPKLDELRDEGKASSAK	K190	trypsin	3
182 - 197	1885.95	LDELRDEGKASSAKQR	K190 K195	trypsin	3
182 - 197	1927.96	LDELRDEGKASSAKQR	K190 S192 K195	trypsin	3
189 - 208	2319.20	GKASSAKQRLKCASLQKFGE	K190 K195 K199	Glu-C	0
189 - 208	2361.21	GKASSAKQRLKCASLQKFGE	K190 K195 K199 K205	Glu-C	0
198 - 209	1519.78	LKCASLQKFGER	K199 K205	trypsin	2
198 - 209	1561.79	LKCASLQKFGER	K199 S202 K205	trypsin	2
210 - 218	1060.58	AFKAWAVAR	K212	trypsin	1
223 - 233	1293.66	FPKAEFAEVSK	K225	trypsin	1
223 - 240	2106.12	FPKAEFAEVSKLVTDLTK	K225 K233	trypsin	2
234 - 257	2898.29	LVTDLTKVHTECCHGDLLECADDR	K240	trypsin	1
245 - 266	2725.12	CCHGDLLECADDRADLAKYICE	K262	Glu-C	1
253 - 266	1740.74	CADDRADLAKYICE	K262	Glu-C	0
258 - 274	1982.93	ADLAKYICENQDSISSK	K262	trypsin	1
258 - 276	2266.12	ADLAKYICENQDSISSKLK	K262 K274	trypsin	2
263 - 276	1725.82	YICENQDSISSKLK	K274	trypsin	1
267 - 277	1289.65	NQDSISSKLKE	K274	Glu-C	0
267 - 277	1331.66	NQDSISSKLKE	K274 K276	Glu-C	0
275 - 286	1629.81	LKECCEKPLLEK	K276 K281	trypsin	2
286 - 292	927.41	KSHCIAE	K286 S287	Glu-C	0
312 - 321	1296.57	SKDVCKNYAE	S312 K317	Glu-C	0
312 - 321	1296.57	SKDVCKNYAE	K313 K317	Glu-C	0
318 - 336	2341.11	NYAEAKDVFLGMFLYEYAR	K323	trypsin	1
322 - 333	1473.72	AKDVFLGMFLYE	K323	Glu-C	0
349 - 359	1337.71	LAKTYETTLEK	K351	trypsin	1
349 - 372	2913.30	LAKTYETTLEKCCAAADPHECYAK	K351 K359	trypsin	2
359 - 376	2211.90	KCCAAADPHECYAKVFDE	K359	Glu-C	1
359 - 376	2253.91	KCCAAADPHECYAKVFDE	K359 K372	Glu-C	1
369 - 376	1072.45	CYAKVFDE	K372	Glu-C	0
377 - 393	2127.07	FKPLVEEPQNLIKQNCE	K378	Glu-C	2
377 - 393	2169.08	FKPLVEEPQNLIKQNCE	K378 K389	Glu-C	2
390 - 410	2640.30	QNCELFEQLGEYKFQNALLVR	K402	trypsin	1
390 - 410	2640.30	QNCELFEQLGEYKFQNALLVR	Y401	trypsin	1
401 - 425	3047.67	YKFQNALLVRYTKKVPQVSTPTLVE	Y401 K402 K413	Glu-C	0
408 - 423	1997.08	LVRYTKKVPQVSTPTL	Y411 K413 K414 S419	pepsin	2
409 - 423	1883.99	VRYTKKVPQVSTPTL	Y411 K413 K414 S419	pepsin	1
411 - 428	2115.16	YTKKVPQVSTPTLVEVSR	K413 K414	trypsin	2
411 - 428	2157.17	YTKKVPQVSTPTLVEVSR	K413 K414 S419	trypsin	2
414 - 428	1680.94	KVPQVSTPTLVEVSR	K414	trypsin	1
414 - 428	1722.95	KVPQVSTPTLVEVSR	K414 S419	trypsin	1
426 - 442	2039.00	VSRNLGKVGSKCCKHPE	K432 K436	Glu-C	0
426 - 442	2081.01	VSRNLGKVGSKCCKHPE	K432 K436 K439	Glu-C	0

426 - 442	2081.01	VSRNLGKVGSKCCKHPE	S427 K436 K439	Glu-C	0
426 - 442	2123.03	VSRNLGKVGSKCCKHPE	K432 S435 K436 K439	Glu-C	0
429 - 439	1333.65	NLGKVGSKCCK	K432 K436	trypsin	2
429 - 444	1937.95	NLGKVGSKCCKHPEAK	K432 K436 K439	trypsin	3
440 - 466	3293.60	HPEAKRM$_{OX}$PCAEDYLSVVLNQLCVLHEK	K444	trypsin	2
446 - 466	2575.21	M$_{OX}$PCAEDYLSVVLNQLCVLHEK	S454	trypsin	0
446 - 472	3214.55	MPCAEDYLSVVLNQLCVLHEKTPVSDR	K466	trypsin	1
446 - 472	3230.54	M$_{OX}$PCAEDYLSVVLNQLCVLHEKTPVSDR	T467	trypsin	1
446 - 472	3256.56	MPCAEDYLSVVLNQLCVLHEKTPVSDR	S454 K466	trypsin	1
466 - 479	1721.81	KTPVSDRVTKCCTE	T467	Glu-C	0
466 - 479	1763.82	KTPVSDRVTKCCTE	K466 K475	Glu-C	0
473 - 484	1507.71	VTKCCTESLVNR	K475	trypsin	1
473 - 484	1549.72	VTKCCTESLVNR	T474 K475	trypsin	1
496 - 505	1238.58	TYVPKEFNAE	K500	Glu-C	1
501 - 521	2586.17	EFNAETFTFHADICTLSEKER	K519	trypsin	1
506 - 520	1839.83	TFTFHADICTLSEKE	K519	Glu-C	1
521 - 531	1396.80	RQIKKQTALVE	K524 K525	Glu-C	0
522 - 534	1580.95	QIKKQTALVELVK	K524 K525	trypsin	2
526 - 534	1041.61	QTALVELVK	T527	trypsin	0
532 - 542	1403.81	LVKHKPKATKE	K534 K538 K541	Glu-C	0
535 - 557	2798.43	HKPKATKEQLKAVMDDFAAFVEK	K536 K538 T540 K545	trypsin	4
539 - 557	2224.11	ATKEQLKAVMDDFAAFVEK	K541 K545	trypsin	2
539 - 560	2772.28	ATKEQLKAVMDDFAAFVEKCCK	T540 K541 K545 K557	trypsin	3
543 - 556	1624.78	QLKAVMDDFAAFVE	K545	Glu-C	0
546 - 560	1847.79	AVMDDFAAFVEKCCK	K557	trypsin	1
558 - 574	2158.90	CCKADDKETCFAEEGKK	K560 K564	trypsin	3
558 - 574	2200.91	CCKADDKETCFAEEGKK	K560 K564 K573	trypsin	3
561 - 573	1540.64	ADDKETCFAEEGK	K564	trypsin	1
561 - 574	1710.74	ADDKETCFAEEGKK	K564 K573	trypsin	2
561 - 585	2705.32	ADDKETCFAEEGKKLVAASQAALGL	K573 K574	trypsin	3
565 - 585	2276.14	ETCFAEEGKKLVAASQAALGL	K573 K574	trypsin	2
572 - 585	1367.81	GKKLVAASQAALGL	K573	Glu-C	0
572 - 585	1409.82	GKKLVAASQAALGL	K573 K574	Glu-C	0
574 - 585	1182.70	KLVAASQAALGL	K574	trypsin	1

The added mass from acetate is 42, and from carbamidomethylation is 57. All 59 lysines are acetylated. 10 serines are acetylated: S5, S65, S192, S202, S287, S312, S419, S427, S435, S454. 8 threonines are acetylated: T68, T76, T79, T83, T467, T474, T527, T540. 4 tyrosines are acetylated: Y84, Y161, Y401, Y411. Each residue listed in the "acetylated" column was unequivocally identified based on the presence of diagnostic b and/or y ions.