

# **Differential Ion Mobility Spectrometry Coupled to Tandem Mass Spectrometry Enables Leukemia Antigen Detection Without HPLC**

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## **Supporting Information:**

1. Supplementary Table S-1: Peptide pool sequences, molecular weights and predicted HLA-A\*0201 binding affinities
2. Supplementary Figure S-1: MS/MS spectra for UNC-CDK4-1 (ALTPVVVTL)
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**Supplementary Table S-1:**

Peptide #	Molecular weight, g/mol	Molecular Formula	Predicted Binding IC <sub>50</sub> to HLA-A*0201 (nM)
<b>GLR</b>	<b>1017.12</b>	<b>H<sub>2</sub>N-FLSSANEHL-OH</b>	<b>19.00</b>
1	1114.37	H <sub>2</sub> N-FLVFFLESL-OH	10.00
2	1146.32	H <sub>2</sub> N-FLKNYFTDV-OH	29.00
3	1189.48	H <sub>2</sub> N-SLGGFLLVFFL-OH	20.00
4	1022.31	H <sub>2</sub> N-LIISLGGFLL-OH	19.00
5	1008.28	H <sub>2</sub> N-IISLGGFLLV-OH	36.00
6	954.24	H <sub>2</sub> N-CILCGLSFV-OH	33.00
7	1051.33	H <sub>2</sub> N-ILCGLSFVSL-OH	71.00
8	1121.44	H <sub>2</sub> N-LIISLGGFLLV-OH	24.00
9	1185.45	H <sub>2</sub> N-FLVFFLESLA-OH	44.00
10	1151.43	H <sub>2</sub> N-ELIISLGGFLL-OH	262.00
11	1075.28	H <sub>2</sub> N-RVFKAVEQV-OH	167.00
12	1075.24	H <sub>2</sub> N-RVFQAVEQV-OH	164.00
13	1004.28	H <sub>2</sub> N-LLLPPSSFM-OH	143.00
14	1224.45	H <sub>2</sub> N-YLFTLEPQTI-OH	13.00
15	1094.35	H <sub>2</sub> N-SLQGFVLMISI-OH	25.00
16	1111.29	H <sub>2</sub> N-YLFTLEPQT-OH	33.00
17	1101.27	H <sub>2</sub> N-TLYWTDCTV-OH	41.00
18	1062.25	H <sub>2</sub> N-LILDAFEEL-OH	32.00
19	965.19	H <sub>2</sub> N-FLGMAVNTL-OH	53.00
20	1175.41	H <sub>2</sub> N-LLILDAFEEL-OH	62.00
21	1287.48	H <sub>2</sub> N-WTLYWTDCTV-OH	131.00
22	1149.29	H <sub>2</sub> N-ILDAFEELEA-OH	110.00
23	1047.39	H <sub>2</sub> N-FIMAAILRI-OH	7.00
24	900.10	H <sub>2</sub> N-SMFAVGCSV-OH	14.00
25	1111.51	H <sub>2</sub> N-LLSLLLLMPV-OH	12.00
26	1076.37	H <sub>2</sub> N-CLLCQPLTSV-OH	31.00
27	986.22	H <sub>2</sub> N-CLTSLLHTV-OH	18.00
28	1028.42	H <sub>2</sub> N-LLSLLLLM-OH	39.00
29	1082.43	H <sub>2</sub> N-IILSFVFIM-OH	71.00
30	1041.29	H <sub>2</sub> N-TLNIHMTLV-OH	84.00
31	973.22	H <sub>2</sub> N-LLCQPLTSV-OH	52.00
32	1156.55	H <sub>2</sub> N-QLLSLLLLM-OH	259.00
33	1052.26	H <sub>2</sub> N-SLPRAAEIPV-OH	62.00
34	1145.47	H <sub>2</sub> N-SLLHTVLTMM-OH	83.00
35	1203.53	H <sub>2</sub> N-LLMPVHPQRL-OH	39.00
36	1153.51	H <sub>2</sub> N-IILSFVFIMA-OH	121.00
37	1014.27	H <sub>2</sub> N-SLLHTVLTM-OH	223.00
38	1040.35	H <sub>2</sub> N-ILSFVFIMA-OH	182.00
39	1101.32	H <sub>2</sub> N-TLLNTGLEKL-OH	122.00
40	1111.43	H <sub>2</sub> N-ILSFVFIMAA-OH	107.00

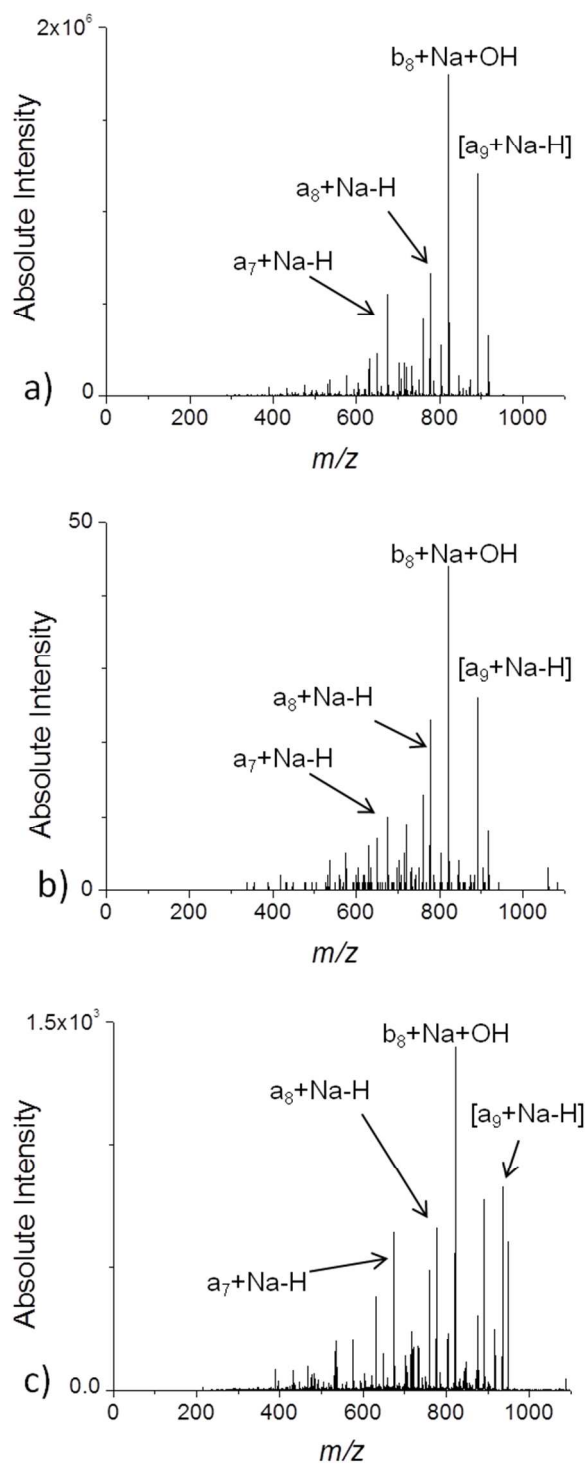
41	1024.35	H <sub>2</sub> N-FVFIMAAIL-OH	295.00
42	1000.21	H <sub>2</sub> N-LLNTGLEKL-OH	155.00
43	1152.29	H <sub>2</sub> N-FTQELEWTV-OH	12.00
44	968.22	H <sub>2</sub> N-AVMAFDLCV-OH	31.00
45	1275.40	H <sub>2</sub> N-QLFYFSEENV-OH	27.00
46	963.09	H <sub>2</sub> N-AMQQQTASV-OH	39.00
47	1110.33	H <sub>2</sub> N-TLGIQFIGYV-OH	114.00
48	1085.29	H <sub>2</sub> N-YLAWLAFTT-OH	17.00
49	1061.25	H <sub>2</sub> N-FTTTLNYCV-OH	31.00
50	1033.16	H <sub>2</sub> N-GLHDHTQLL-OH	34.00
51	1186.40	H <sub>2</sub> N-YLAWLAFTTT-OH	45.00
52	1048.20	H <sub>2</sub> N-SAMSYDLYV-OH	14.00
53	1032.20	H <sub>2</sub> N-AMSYDLYVA-OH	50.00
54	1159.39	H <sub>2</sub> N-FILSAMSVDL-OH	27.00
55	1012.21	H <sub>2</sub> N-ILSAMSVDL-OH	74.00
56	1119.28	H <sub>2</sub> N-SAMSYDLYVA-OH	219.00
57	1145.36	H <sub>2</sub> N-AMSYDLYVAI-OH	91.00
58	1200.55	H <sub>2</sub> N-IMFRHVCVPV-OH	10.00
59	1132.47	H <sub>2</sub> N-KIMFRHVCV-OH	105.00
60	977.25	H <sub>2</sub> N-FIGSKLCPL-OH	24.00
61	1099.18	H <sub>2</sub> N-SLPDDDLPEV-OH	15.00
62	983.23	H <sub>2</sub> N-IMNSCVFGI-OH	7.00
63	1054.31	H <sub>2</sub> N-AIMNSCVFGI-OH	16.00
64	1221.50	H <sub>2</sub> N-MLQVFRDISL-OH	125.00
65	1104.32	H <sub>2</sub> N-GLQAIDLVI-OH	56.00
66	1047.27	H <sub>2</sub> N-LQAIDLVI-OH	348.00
67	963.21	H <sub>2</sub> N-AMNGVIFLV-OH	4.00
68	1123.40	H <sub>2</sub> N-PQMEIIFV-OH	8.00
69	1187.51	H <sub>2</sub> N-MVTEFIFMGI-OH	98.00
70	1078.27	H <sub>2</sub> N-NLLGSEPFAM-OH	109.00
71	947.07	H <sub>2</sub> N-NLLGSEPF-A-OH	112.00
72	964.17	H <sub>2</sub> N-LLGSEPFAM-OH	180.00
73	1168.46	H <sub>2</sub> N-FMLLIEQSGM-OH	68.00
74	905.05	H <sub>2</sub> N-VAADCLTNV-OH	161.00
75	992.13	H <sub>2</sub> N-SVAADCLTNV-OH	87.00
76	1173.39	H <sub>2</sub> N-FLGTKHNPF-I-OH	36.00
77	1141.42	H <sub>2</sub> N-LVLELKNLTV-OH	127.00
78	972.11	H <sub>2</sub> N-VLPGSSSLNV-OH	139.00
79	926.09	H <sub>2</sub> N-GLADLLEPV-OH	3.00
80	1045.22	H <sub>2</sub> N-LLSDIPSST-OH	135.00
81	1052.12	H <sub>2</sub> N-SLDEASGFNI-OH	42.00
82	1248.59	H <sub>2</sub> N-GMFLMLFYNI-OH	23.00
83	892	H <sub>2</sub> N-TLVESTATA-OH	71.00
84	1175.42	H <sub>2</sub> N-KLSWFNHML-OH	11.00
85	997.21	H <sub>2</sub> N-VLAALVESPV-OH	19.00

86	978.17	H <sub>2</sub> N-SIYTPVVSL-OH	184.00
87	964.14	H <sub>2</sub> N-SVSIYTPVV-OH	113.00
88	1179.50	H <sub>2</sub> N-IMIYWDLI-OH	116.00
89	1165.47	H <sub>2</sub> N-VIMIYWDL-OH	507.00
90	1127.24	H <sub>2</sub> N-AVFDRYEEV-OH	37.00
91	1006.27	H <sub>2</sub> N-ILAFYIPGL-OH	4.00
92	1105.40	H <sub>2</sub> N-VILAFYIPGL-OH	29.00
93	1130.45	H <sub>2</sub> N-LLGYAVLRL-OH	20.00
94	1335.62	H <sub>2</sub> N-RLRHWWVIAV-OH	85.00

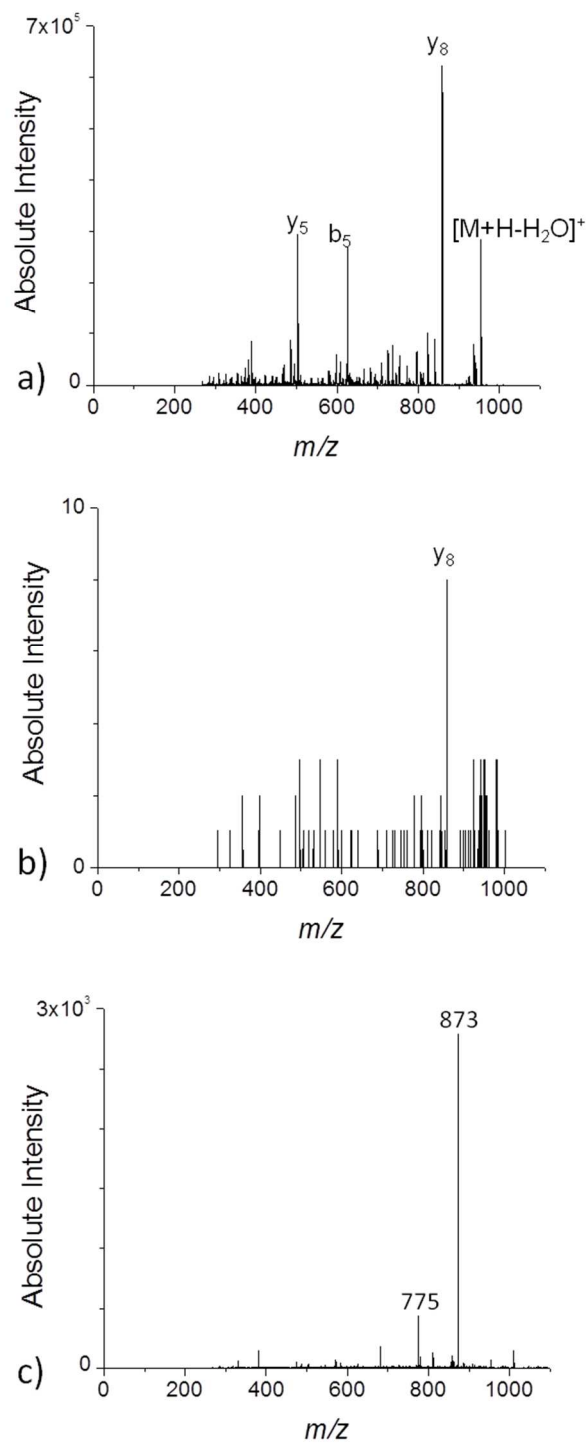
**Table S-1. Peptide pool sequences, molecular weights and predicted HLA-A\*0201 binding affinities.** A genomics screen was used to predict a series of potential antigens based upon their predicted binding affinity to HLA-A\*0201. Binding predictions were performed using the IEDB analysis resource ANN aka netMHC.<sup>1,2</sup> Peptides with a predicted binding affinity of < 50 nM are considered tight binders, while peptides with predicted binding affinity of between 50 and 500 nM are considered moderate binders.

**References:**

1. Lundegaard C, Lamberth K, Harndahl M, Buus S, Lund O, Nielsen M. NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11. *Nucleic Acids Res* 2008;36:W509-12.
2. Nielsen M, Lundegaard C, Worning P, et al. Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. *Protein Sci* 2003;12:1007-17.



**Figure S-1. MS/MS spectra for UNC-CDK4-1 (ALTPVVVTL).** (a) was obtained for pure 10  $\mu\text{M}$  ALTPVVVTL in 50/49.9/0.1 acetonitrile/water/formic acid, (b) was obtained with DIMS active selecting for protonated ALTPVVVTL in a leukemia cell extract, and (c) was obtained without DIMS for the same leukemia cell extract.



**Figure S-2. MS/MS spectra for UNC-ANKRD17-1 (LLIERGASL).** (a) was obtained for pure 10  $\mu\text{M}$  LLIERGASL in 50/49.9/0.1 acetonitrile/water/formic acid, (b) was obtained with DIMS active selecting for protonated LLIERGASL in a leukemia cell extract, and (c) was obtained without DIMS for the same leukemia cell extract.