Title: Production and characterization of a camelid single domain antibody-urease enzyme conjugate for the treatment of cancer

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SUPPORTING INFORMATION

The LC-MS TIC chromatograms of the tryptic digests of HP urease and L-DOS47 conjugate are shown in Figure S1.

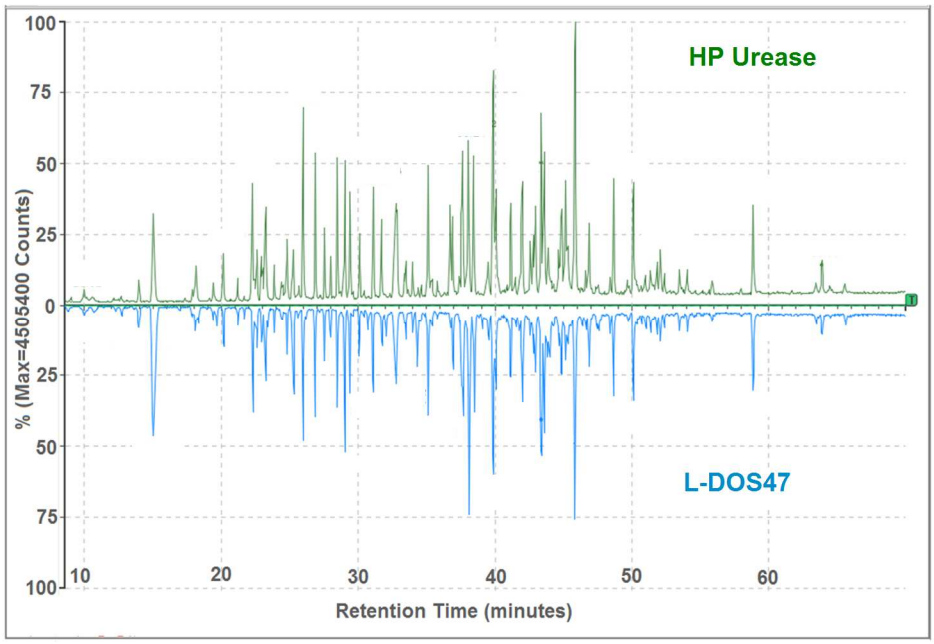

Figure S1. LC-MS TIC raw chromatograms of the tryptic digests of L-DOS47 and HPU reference standards

The deconvoluted spectra of the tryptic digests of HP urease and L-DOS47 conjugate are shown in Figure S2.

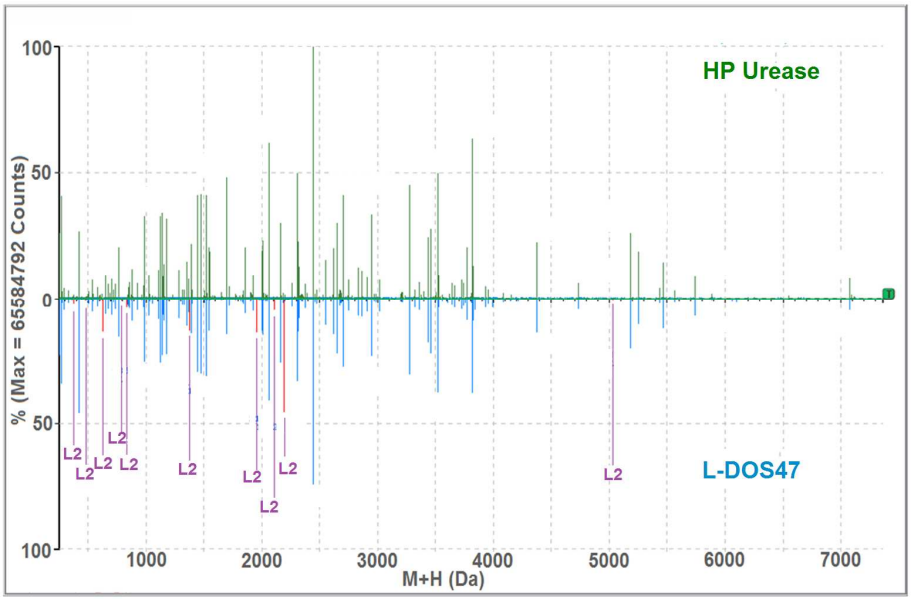

Figure S2. Deconvoluted spectra of L-DOS47 and HPU reference standards. AFAIKL2 peptide peaks are highlighted in red, and labeled with L2

As shown in Figure S2, tryptic peptides of AFAIKL2 were identified in the spectrum of L-DOS47, but not from the urease sample. The identified AFAIKL2 tryptic peptides are listed in Table S1.

Table S1. List of AFAIKL2 tryptic peptides identified in L-DOS47

Peptide #	RT (min)	Calculated mass (Da)	Detected mass (Da)	Match error (ppm)	Intensity (counts)	*b/y* possible	*b/y* found
LT1	32.9	1954.9894	1954.99	0.3	9195867	38	29
LT2*	27.6	1372.6445	1372.6453	0.6	8755624	22	22
LT2-3*	36.7	2187.0354	2187.0408	2.5	29977870	36	28
LT3	30.6	832.4014	832.4015	0.1	2695649	12	10
LT4	3.1	499.2754	499.2766	2.4	214874	8	4
LT4-5	3.1	783.4351	783.4371	2.6	831944	12	7
LT5	13.3	302.1703	302.169	-4.3	91456	2	2
LT5-6	28	2388.1306	2388.1321	0.6	220247	44	17
LT6	29.2	2103.9709	2103.9719	0.5	3300428	40	35
LT7	23.8	622.3439	622.3436	-0.5	9092843	8	7
LT7-8	21.8	1050.5458	1050.5465	0.7	1734182	16	8
LT9-10*	42.3	5028.3071	5028.3032	-0.8	979526	90	24
LT10*	44.2	4900.2119	4900.2217	2	639656	88	33

* denotes the peptides carrying an alkylated cysteine residue

As shown in Table S1, the AFAIKL2 peptides identified from the tryptic digest of the L-DOS47 sample covered 100% of the amino acid sequence of the antibody. The mass match errors of all the identified antibody peptide were less than ±5 ppm. All identified peptides were confirmed by their respective MS/MS *b/y* fragment ions. The urease tryptic peptides identified in the L-DOS47 sample are listed in Table S2

Table S2. List of urease tryptic peptides identified in L-DOS47

Peptide #	RT (Min)	Calculated Mass (Da)	Detected Mass (Da)	Error (ppm)	Intensity (Counts)	b/y Possible	b/y Found
UT1	16.8	277.146	277.1456	-1.4	215424	2	1
UT1-2	20.6	730.416	730.4163	0.4	3046560	10	6

UT2	27.5	471.2805	471.2801	-0.8	102514	6	3
UT3	3.2	503.2591	503.2604	2.6	343043	6	5
UT3-4	27.8	1768.9471	1768.9495	1.4	820529	30	7
UT4	27	1283.6986	1283.6987	0.1	5451383	22	21
UT4-5	25.4	1439.7997	1439.8016	1.3	19415200	24	20
UT5	3.1	174.1117	174.1121	2.3	185620	0	0
UT6	4.1	358.2328	358.2337	2.5	427821	4	3
UT7	3.1	330.2015	330.2017	0.6	340523	4	3
UT7-8	54.5	2467.2893	2467.2893	0	1654470	42	26
UT8	57.4	2155.0984	2155.0977	-0.3	17099280	36	32
UT8-9	53.6	2584.2842	2584.2817	-1	32022	44	4
UT9-10*	45.7	2125.0771	2125.084	3.2	311268	36	9
UT10*	43.8	1695.8912	1695.8953	2.4	9625287	28	20
UT10-11*	41.2	1851.9924	1851.9933	0.5	4125558	30	14
UT11-12	42	2699.4758	2699.479	1.2	18284950	48	38
UT12	44.5	2543.3748	2543.3796	1.9	7252773	46	37
UT12-13	45.3	3660.999	3661.0071	2.2	2623853	66	29
UT13	27.5	1135.6349	1135.6351	0.2	15079610	18	13
UT13-14	48.4	3372.7927	3372.7949	0.7	766619	60	22
UT14	50.5	2255.1685	2255.1711	1.2	2508607	40	26
UT14-15	50.7	2831.4592	2831.4609	0.6	4840426	50	34
UT15	11.9	594.3013	594.3009	-0.7	482062	8	5
UT16-17*	40.6	2615.2319	2615.2388	2.6	5389101	42	29
UT17*	41	2101.0183	2101.0242	2.8	75750	34	2
UT17-18*	38.7	2229.1133	2229.1189	2.5	47040	36	2
UT18	20.6	146.1055	146.1053	-1.4	70287	0	0

UT18-19	20.6	670.4741	670.4739	-0.3	3162423	10	10
UT19	23.6	542.3792	542.379	-0.4	988100	8	6
UT19-20	26.3	957.6223	957.6226	0.3	64469	16	3
UT20-021	37.1	3274.6523	3274.6609	2.6	20158930	54	33
UT21	38.4	2859.4092	2859.4136	1.5	5008401	46	21
UT21-22	36.8	3015.5103	3015.52	3.2	3660867	48	19
UT23-24	12.3	724.369	724.366	-4.1	512591	10	6
UT24	16.4	596.274	596.2729	-1.8	636494	8	5
UT25	27.9	984.5716	984.5721	0.5	16833270	18	16
UT26*	18.5	851.3484	851.3472	-1.4	2855264	12	8
UT27	31.3	1145.6292	1145.6305	1.1	4071953	20	12
UT27-28	33.6	1513.8828	1513.8861	2.2	20701940	26	17
UT27-28/y12	33.6	1327.7823	1327.7841	1.4	1698112	22	20
UT28	6	386.2641	386.2652	2.8	407633	4	4
UT28-29	35.9	2674.3608	2674.3689	3	1556611	50	26
UT29	36.6	2306.1072	2306.1104	1.4	21967850	44	27
UT29-30*	54.5	2520.2026	2520.2034	0.3	37031	48	0
UT29/y18	36.6	1893.9003	1893.903	1.4	32988	34	9
UT31-32	22.8	1766.7747	1766.774	-0.4	677463	30	12
UT32	19	853.3818	853.3816	-0.2	40675	14	4
UT32-33*	30.3	2464.0754	2464.0874	4.9	94726	40	16
UT33*	33.7	1645.7307	1645.7313	0.4	235711	24	8
UT33-34*	31.1	1773.8257	1773.8281	1.4	445610	26	9
UT35	3.8	623.2915	623.2934	3	172138	8	7
UT35-36	20	1442.6677	1442.6702	1.7	281669	24	10
UT36	15.7	837.3868	837.3864	-0.5	513823	14	6

UT36-37	20.9	1106.572	1106.5732	1.1	5656579	18	11
UT37	6	287.1957	287.1957	0	1111399	2	1
UT38	38.5	1314.7031	1314.7036	0.4	1900970	22	15
UT38-39*	47.3	2946.3958	2946.396	0.1	15314400	52	42
UT39*	34.3	1649.7031	1649.7067	2.2	603856	28	18
UT39-40*	35.9	2017.9568	2017.9648	4	161223	34	11
UT40-41*	50	3723.9329	3723.9392	1.7	3163381	68	37
UT41*	52.1	3355.6792	3355.6797	0.1	3201687	62	40
UT42	22.9	615.3591	615.3579	-2	864115	10	7
UT42-43	36.3	1469.8453	1469.8477	1.6	19962440	28	25
UT43	31.4	872.4967	872.4977	1.1	1494874	16	7
UT44*	62.4	7067.4927	7067.501	1.2	3221439	138	42
UT45*	19.7	1365.6017	1365.6003	-1	74329	22	3
UT43	31.4	872.4967	872.4977	1.1	1494874	16	7
UT44*	62.4	7067.4927	7067.501	1.2	3221439	138	42
UT45*	19.7	1365.6017	1365.6003	-1	74329	22	3
UT45-H2O*	23.2	1331.5962	1331.5955	-0.5	382569	22	14
UT46	45.5	1995.9976	1995.9988	0.6	8829908	34	30
UT46-47	42.3	3516.781	3516.782	0.3	25240900	62	36
UT47	27	1538.7939	1538.7944	0.3	8678479	26	22
UT48	21.3	646.3472	646.3468	-0.6	4460865	12	10
UT49*	43.5	5250.5103	5250.5259	3	7065667	92	45
UT49-50*	42.5	5463.6328	5463.6309	-0.3	8125789	96	42
UT51	24.5	2060.0076	2060.0142	3.2	27002440	38	31
UT51-52*	28.6	2617.3071	2617.311	1.5	9578254	48	28
UT51	24.5	2060.0076	2060.0142	3.2	27002440	38	31

UT51-52*	28.6	2617.3071	2617.311	1.5	9578254	48	28
UT52*	16.6	575.3101	575.3096	-0.9	2338988	8	7
UT52-53*	38.7	4370.1392	4370.1494	2.3	9171064	74	24
UT52-53-H2O*	38.7	4352.1289	4352.1421	3	117039	74	30
UT53*	38.5	3812.8396	3812.8452	1.5	25111560	64	34
UT53-54*	40.7	5178.5073	5178.5244	3.3	13270920	88	25
UT54	31.3	1383.6782	1383.6805	1.7	9339997	22	11
UT54-55	44.5	1652.8634	1652.8846	12.8	27689	26	1
UT54/y10	31.3	1141.5516	1141.5531	1.3	4536739	18	14
UT57-58	46	2774.4119	2774.4138	0.7	721132	52	30
UT58	48.7	2646.3169	2646.3188	0.7	14602610	50	40
UT58-59*	36.1	3402.73	3402.759	8.5	29448	64	1
UT59	21.6	758.4286	758.4279	-0.9	10335030	12	11
UT60	17.8	848.4028	848.4038	1.2	2416820	12	8
UT60-61	21.4	1107.5383	1107.5372	-1	3628896	16	8
UT61-62	20.5	972.5426	972.5441	1.5	159410	16	2
UT62	18.5	713.4072	713.4058	-2	1264002	12	8
UT62-63*	23.7	1923.8381	1923.8374	-0.4	1208402	32	16
UT63*	16.5	1228.4415	1228.4399	-1.3	271614	18	10
UT63-64*	23.9	1497.6267	1497.627	0.2	49237	22	1
UT64-65	2.9	443.2968	443.2982	3.2	299037	4	1
UT65-66	10.1	649.3911	649.3908	-0.5	397808	8	3
UT66	9.9	493.29	493.2902	0.4	1087679	6	5
UT67	41.6	2313.1641	2313.1643	0.1	9097182	42	35
UT67-68	52.7	4731.4482	4731.4561	1.7	2962509	84	42
UT68	44.5	2436.2949	2436.3013	2.6	48817880	40	25

UT68-69	49	4739.4204	4739.4219	0.3	267230	86	9
UT69	41.5	2321.1362	2321.1372	0.4	5395435	44	33
UT69-70	42.1	3455.7039	3455.707	0.9	14697710	64	36
UT69/b16	41.5	1554.7136	1554.7144	0.5	236449	30	20
UT70	26	1152.5784	1152.5775	-0.8	6083278	18	17
UT71	36.1	1119.6288	1119.6309	1.9	17202190	22	18
UT72	13.3	672.3555	672.3539	-2.4	2183400	10	8
UT72-73	34.4	1672.926	1672.9297	2.2	337096	28	6
UT73	32.1	1018.5811	1018.5817	0.6	4811210	16	12
UT73-74	29.6	1174.6823	1174.682	-0.3	14919070	18	13
UT74-75	19.5	1028.5726	1028.5725	-0.1	1026015	16	16
UT75	20.6	872.4716	872.4714	-0.2	6098755	14	10
UT75-76	18.5	1000.5665	1000.565	-1.5	573108	16	8
UT76-77	3.2	488.3322	488.3338	3.3	592675	6	4
UT77	3.2	360.2373	360.2373	0	34479	4	2
UT77-78	21.6	847.4837	847.4822	-1.8	44872	12	7
UT78	16.8	505.257	505.257	0	1664216	6	4
UT78-79	42.7	2490.2563	2490.2563	0	595609	42	13
UT79	39.8	2003.0099	2003.0146	2.3	9900395	34	27
UT79-80	38.1	2374.1904	2374.1914	0.4	2844146	42	27
UT80-81*	34.3	1916.0037	1916.0094	3	86256	34	6
UT81*	35.3	1544.8232	1544.8248	1	783060	26	15
UT82	43.5	702.3377	702.3387	1.4	4509405	8	7

*denotes peptides carrying alkylated cysteine

As shown in Table S2, the amino acid sequence recovery of urease is also 100%.

To identify those covalently cross-linked peptides, ESI LC-MS^E raw data of the L-DOS47 tryptic digests were processed by BiopharmaLynx and searched against a variable-modifier library containing a set of user-created modifiers for all 15 cysteine residues on the urease side. According to the activation distribution in Table 1 in the main text, those user created modifiers were the three lysine-in-middle peptides plus the linkage portion of SIAB ($C_9H_5O_2N$, 159.0320Da) (denoted as $L2K_{76}$, $L2K_{44}$ and $L2K_{32}$), and the N-terminal methionine plus the linkage (denoted as $L2M_1$). The ion intensity and the intensity % of the identified conjugated peptides are listed in Table S3. All the low energy MS peptide mass match errors are less than 6ppm (not listed).

Table S3. Summary of conjugation sites

Peptide #	Urease Cys#	AFAIKL2 L-side	L-DOS47 (control)		L-DOS47 (Agitation)		L-DOS47 (Freeze/thaw)	
			Intensity	%	Intensity	%	Intensity	%
UT10	UC59	Total	2.54E+07	100.0	2.34E+07	100.0	2.07E+07	100.0
		L2M1	2.63E+06	**10.4**	2.33E+06	**9.9**	1.87E+06	**9.0**
		L2K44	1.30E+06	**5.1**	1.27E+06	**5.4**	1.17E+06	**5.7**
		L2K76	2.15E+06	**8.5**	1.78E+06	**7.6**	1.63E+06	**7.9**
		L2K32	3.66E+06	**14.4**	2.80E+06	**12.0**	2.71E+06	**13.1**
UT17	UC139 and UC143	Total	8.46E+06	100.0	5.96E+06	100.0	5.28E+06	100.0
		L2M1	4.21E+04	0.5	2.87E+04	0.5	1.89E+04	0.4
		L2K44	2.26E+05	2.7	1.44E+05	2.4	6.61E+04	1.3
		L2K76	1.13E+05	1.3	6.12E+04	1.0	4.23E+04	0.8
		L2K32	1.48E+05	1.7	1.15E+05	1.9	1.07E+05	2.0
UT26	UC207	Total	5.96E+06	100.0	5.97E+06	100.0	5.46E+06	100.0
		L2M1	1.14E+05	1.9	8.74E+04	1.5	8.00E+04	1.5
		L2K44	2.20E+05	3.7	2.76E+05	4.6	2.31E+05	4.2
		L2K76	3.33E+05	**5.6**	3.27E+05	**5.5**	2.98E+05	**5.5**
		L2K32	5.97E+05	**10.0**	5.82E+05	**9.7**	5.14E+05	**9.4**
UT33	UC268	Total	2.02E+06	100.0	1.81E+06	100.0	1.70E+06	100.0
		L2M1	1.05E+05	5.2	5.39E+04	3.0	5.12E+04	3.0
		L2K44	1.29E+04	0.6	0.00E+00	0.0	4.18E+03	0.2
		L2K76	5.17E+04	2.6	3.28E+04	1.8	4.11E+04	2.4
		L2K32	1.91E+05	**9.5**	1.74E+05	**9.6**	1.36E+05	**8.0**
UT39	UC313	Total	1.81E+07	100.0	1.71E+07	100.0	1.54E+07	100.0
		L2M1	3.59E+05	2.0	3.66E+05	2.1	3.13E+05	2.0
		L2K44	3.11E+04	0.2	1.55E+04	0.1	5.81E+03	0.0
		L2K76	1.51E+04	0.1	1.49E+04	0.1	0.00E+00	0.0
		L2K32	0.00E+00	0.0	0.00E+00	0.0	0.00E+00	0.0
UT41	UC329	Total	1.16E+07	100.0	1.14E+07	100.0	9.14E+06	100.0
		L2M1	1.57E+06	**13.6**	1.74E+06	**15.3**	1.43E+06	**15.7**
		L2K44	8.77E+03	0.1	0.00E+00	0.0	0.00E+00	0.0
		L2K76	3.82E+03	0.0	5.50E+03	0.0	0.00E+00	0.0
		L2K32	0.00E+00	0.0	0.00E+00	0.0	0.00E+00	0.0

UT44	UC406 and UC412	Total	3.77E+06	100.0	4.53E+06	100.0	3.67E+06	100.0
		L2M1	0.00E+00	0.0	0.00E+00	0.0	0.00E+00	0.0
		L2K44	0.00E+00	0.0	0.00E+00	0.0	0.00E+00	0.0
		L2K76	1.63E+04	0.4	2.21E+04	0.5	0.00E+00	0.0
		L2K32	0.00E+00	0.0	0.00E+00	0.0	0.00E+00	0.0
UT45	UC443	Total	1.17E+07	100.0	1.09E+07	100.0	9.60E+06	100.0
		L2M1	1.34E+05	1.2	2.98E+04	0.3	8.04E+04	0.8
		L2K44	2.47E+05	2.1	2.22E+05	2.0	2.05E+05	2.1
		L2K76	5.25E+05	4.5	4.28E+05	3.9	4.06E+05	4.2
		L2K32	1.98E+05	1.7	1.12E+05	1.0	1.39E+05	1.4
UT52	UC561	Total	2.32E+07	100.0	2.42E+07	100.0	2.20E+07	100.0
		L2M1	1.86E+04	0.1	0.00E+00	0.0	8.97E+04	0.4
		L2K44	0.00E+00	0.0	4.56E+04	0.2	1.40E+04	0.1
		L2K76	0.00E+00	0.0	0.00E+00	0.0	0.00E+00	0.0
		L2K32	0.00E+00	0.0	0.00E+00	0.0	0.00E+00	0.0
UT53	UC591*	Total	5.01E+07	100.0	4.92E+07	100.0	4.50E+07	100.0
		L2M1	5.77E+04	0.1	2.70E+04	0.1	2.61E+04	0.1
		L2K44	1.29E+05	0.3	7.21E+04	0.1	3.34E+04	0.1
		L2K76	0.00E+00	0.0	0.00E+00	0.0	0.00E+00	0.0
		L2K32	0.00E+00	0.0	2.54E+04	0.1	1.89E+04	0.0
UT63	UC663	Total	5.77E+06	100.0	5.38E+06	100.0	4.83E+06	100.0
		L2M1	3.64E+05	**6.3**	3.15E+05	**5.9**	2.95E+05	**6.1**
		L2K44	1.18E+06	**20.4**	1.06E+06	**19.8**	9.97E+05	**20.6**
		L2K76	4.79E+05	**8.3**	4.20E+05	**7.8**	4.04E+05	**8.4**
		L2K32	1.71E+06	**29.7**	1.56E+06	**29.0**	1.33E+06	**27.4**
UT81	UC824	Total	9.01E+06	100.0	8.26E+06	100.0	7.29E+06	100.0
		L2M1	1.93E+06	**21.4**	1.57E+06	**19.1**	1.32E+06	**18.1**
		L2K44	5.04E+05	**5.6**	4.57E+05	**5.5**	4.31E+05	**5.9**
		L2K76	1.00E+06	**11.1**	8.63E+05	**10.5**	8.13E+05	**11.2**
		L2K32	3.95E+06	**43.8**	3.67E+06	**44.4**	3.23E+06	**44.3**

UC591* denotes the urease peptide with the cysteine critical to enzyme activity.
L-DOS47-Control is L-DOS47 reference standard, L-DOS47-Agitation is the L-DOS47 reference standard agitated by stirring overnight at 4ºC, and the L-DOS47-Freeze/Thaw is the reference standard subjected to 5 cycles of freeze(-80ºC)/thaw(4ºC). The conjugation site identifications are consistent among the three samples suggesting that the identification of conjugation sites by this approach is reasonably reproducible because the stresses by agitation and freeze/thaw should not affect the conjugation sites.

The "Total Intensity" is the intensity sum of all the related tryptic peptides including the 1-site missing-cleaved UTX.$_{-1}$-X, the UTX, the 1-site missing-cleaved UTX-X$_{+1}$ and the respective conjugated peptides.

The "intensity % of conjugated peptides" was calculated as shown below:

% = 100* (conjugated peptide intensity)/(Total intensity)

Though the term "intensity % of conjugated peptide" does not accurately represent the conjugated amount of each conjugation site compared to its total peptide amount because the charge properties and masses of the $L2K_x$(or M_1) (where x=32, 44 and 76) are so different that the ESI ionization sensitivities are very different each other, trends in conjugation can be evaluated (Table S3). As shown in Table 1 in the main text, ~18% of $L2K_{32}$ was activated by the cross-linker, however, $L2K_{32}$ was the most sensitive antibody site linking to urease UCx (where x =824, 663, 59, 268 and 267) to be detected as shown in much higher percentages comparing to that of the other antibody sites. This is most likely due to the alkylation of the cysteine in $L2K_{32}$ which introduced an amide group, thus increasing the ESI ionization sensitivity. The detected conjugation percentage of each conjugation site represented only the trend for each urease-cysteine site (UC_x) to be linked by activated AFAIKL2. As shown in Table S3, among the 15 cysteine residues of each urease subunit, only 6 were substantially conjugated. The most accessible cysteine is UC_{824} followed in order by UC_{663}, UC_{59}, UC_{207}, UC_{329} and UC_{268}. Cys_{592}, which is essential to urease enzyme activity, was not substantially conjugated. The relative accessibilities of the four cross-linker activated AFAIKL2 sites to each of the six cysteine residues on the urease side were also different. For example, UC_{329} was only accessible to $L2M_1$. Each conjugated peptide with intensity greater than 10% was confirmed by at least *3 b/y* fragment ions (mass match error less than 15 ppm) from its correspondent MS^E MS/MS fragment spectrum. For example, the conjugated peptide L2K32UC663, whose sequence is (LSCAAHDPIFDKNLMGWGR)-linkage-(CDSSDNDNFR) and which has a peptide mass of 3517.4873, was identified with a mass match error of 2.1ppm by searching it as CDSSDNDNFR a urease peptide modified with (LSCAAHDPIFDKNLMGWGR)-linkage (2346.0674Da) from the AFAIKL2 side as the modifier. The same peptide was also identified with a mass match error of 2.1ppm by searching it as LSCAAHDPIFDKNLMGWGR a AFAIKL2 peptide modified with the linkage-(CDSSDNDNFR) (1330.4520 Da) from the urease side as the modifier. The MS^E MS/MS spectrum of this conjugated peptide was mapped with 9 *b/y* fragment ions from the urease side by searching it as a urease peptide modified with the modifier from the AFAIKL2 side shown in Figure S3 (panel A). The same MS/MS spectrum was also mapped with 14 *b/y* ions from the AFAIKL2 side by searching it as an AFAIKL2 peptide with the modifier from the urease side (Figure S3, panel B).

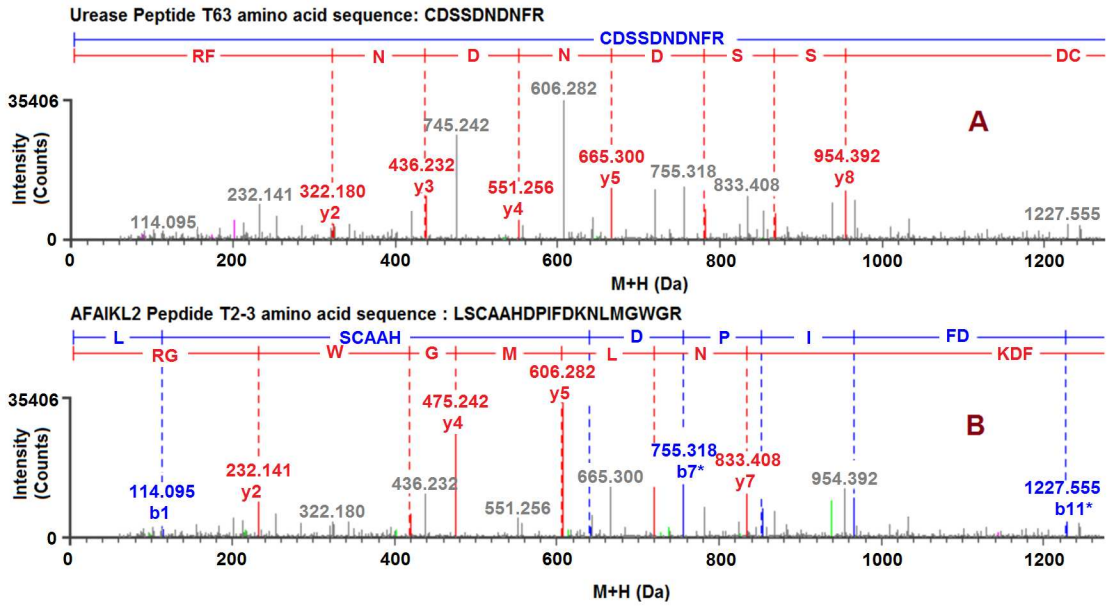

Figure S3. Part of the MS^E MS/MS fragment map of conjugate site L2K32UC663. A: Search the spectrum as of the urease peptide with the cross-linked AFAIKL2 peptide as the modifier. B: Search the same spectrum as of the AFAIKL2 peptide with the cross-linked urease peptide as the modifier. The *y* ion peaks are highlighted in red, and the *b* ion peaks are highlighted in blue. The gray peaks are not identified.

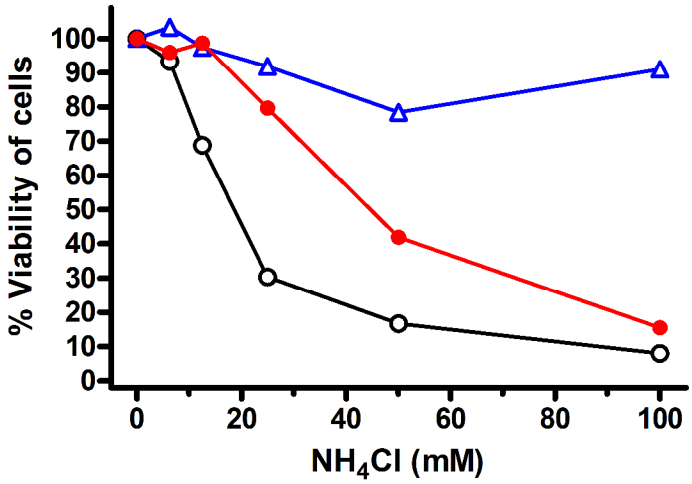

Figure S4. Cytotoxicity of ammonium chloride on A549, BxPC-3, and H23 cells. Cell monolayers of the three cell lines were treated with 100µL/well of serially diluted NH_4Cl solution in KR-II buffer, pH 7.4. The plate was incubated at 37°C overnight and cell viability was determined with MTS cell viability assay. The H23 cells (○) was found to be more sensitive to NH_4Cl toxicity as compared to BxPC-3 (●) and A549 (△).The results represent the mean (n=3) of representative experiments. The standard deviation (SD) was less than 10% for all values.

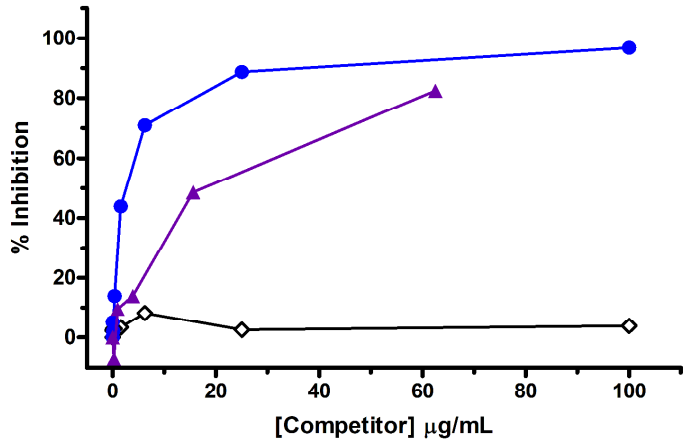

Figure S5. Competitive binding assays of L-DOS47, DOS47, and AFAIKL2 antibody to BxPC-3 cells. The electrochemiluminescence assay was employed to provide a direct measurement of ruthenium-tagged L-DOS47 binding to BxPC-3 cells. The binding was competed with either L-DOS47, AFAIKL2 antibody, or DOS47 in order to determine and compare their relative binding affinities. The apparent binding affinities of both L-DOS47 and AFAIKL2 antibody can be determined from the respective IC_{50} (the amount of competitor required to cause 50% decrease in binding) of the test articles. The IC_{50} of L-DOS47 (●) and AFAIKL2 antibody (▲) were estimated as 2 and 20μg/mL (or 3.22nM and 1.55μM), respectively, indicating that the binding affinity of L-DOS47 is about 500 times of that of AFAIKL2 antibody. No inhibition was observed with the negative control DOS47 (◇). The results represent the mean (n=3) of representative experiments. The standard deviation (SD) was less than 10% for all values.