Supporting information for "A multiple reaction monitoring (MRM) method activity in CML using a peptide biosensor," Yang of	d to detect Bcr-Abl kinase et al.

Figure S1. LC/MS characterization of Abl biosensor peptide. Used in assays and also spiked into K562 lysate for digestion and use as MRM calibration standard for EAIYAAPFAK

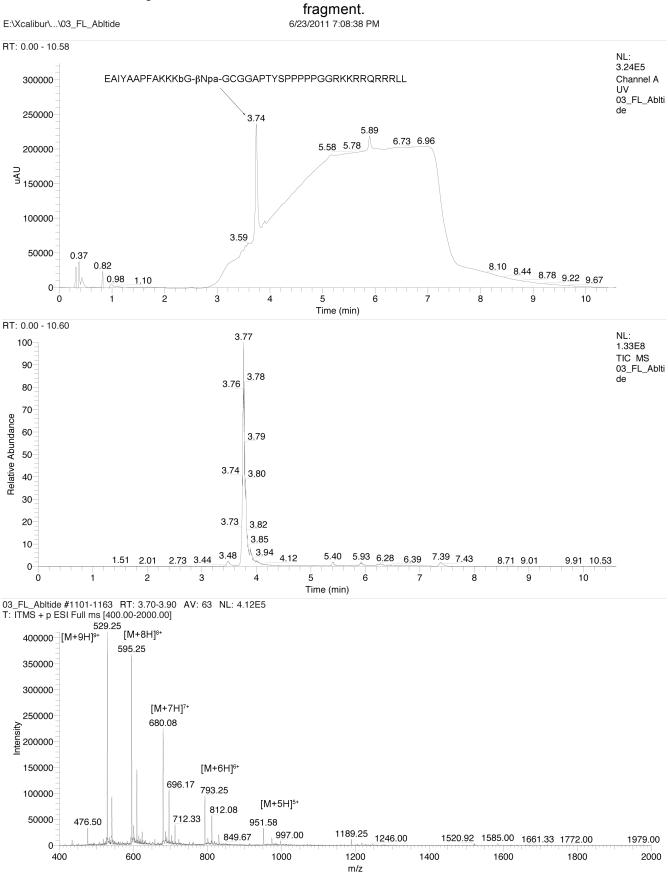
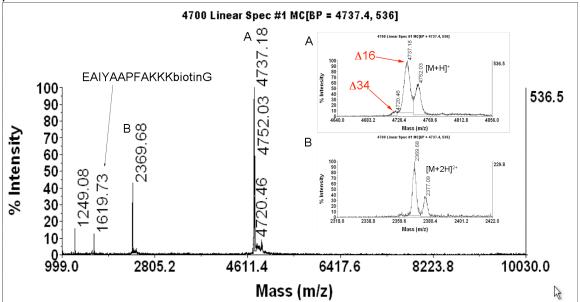


Figure S2. MALDI-TOF characterization of Abl biosensor; shows what appear to be laser-induced photolytic degradation peaks that may represent the intermediates proposed in Scheme S1.¹⁻² While the resolution at this mass range makes the m/z differences appear to be 15-17 and 32-34, similar peaks in spectra from other fragments (including Fig. S5 and Fig. S6) show that these losses are more likely to be 16 and 34, and these values are consistent with a plausible proposed mechanism for their formation (Scheme 1). Photolysis peaks are not observed in the LC/ESI-MS spectrum given in S1, indicating that this is a laser desorption mediated process.



1. Gaplovsky, M.; Il'ichev, Y. V.; Kamdzhilov, Y.; Kombarova, S. V.; Mac, M.; Schworer, M. A.; Wirz, J., Photochemical reaction mechanisms of 2-nitrobenzyl compounds: 2-Nitrobenzyl alcohols form 2-nitroso hydrates by dual proton transfer. *Photoch Photobio Sci* **2005**, *4* (1), 33-42.

2. Il'ichev, Y. V.; Schworer, M. A.; Wirz, J., Photochemical reaction mechanisms of 2-nitrobenzyl compounds: Methyl ethers and caged ATP. *Journal Of The American Chemical Society* **2004**, *126* (14), 4581-4595.

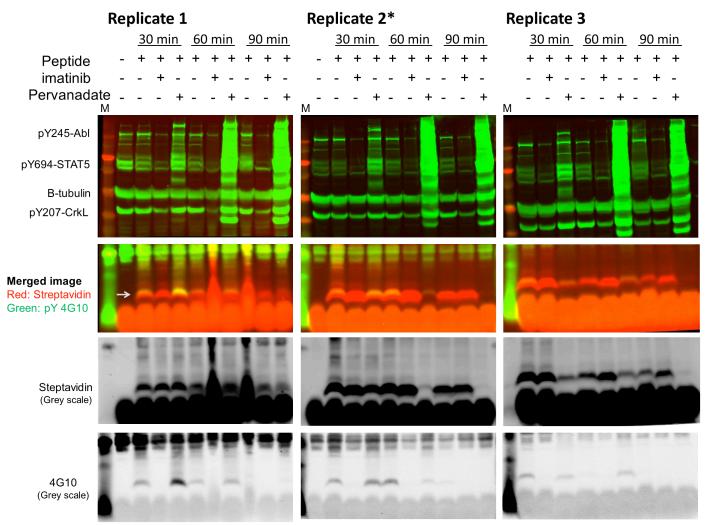
Scheme S1. Proposed potential photodegradation intermediates (as observed in MALDI-TOF spectra). The observed ions exhibit m/z values of 16 amu (major) and 34 amu (minor) less than the expected m/z. The major species is likely formed via gas phase ion chemistry involving protonation of one of the oxygens in the nitro group by initial photo-tautomerization (which is well-characterized in the literature, see 1. and 2. above), additional protonation by acid and elimination as water. For the minor product, the second loss of water (18 amu) to give a total net loss of 34 amu overall could occur—however, the mechanism for this is not clear and while it is very interesting, its absolute determination would be outside the scope of this work. However, this phenomenon suggests that MALDI-MS might be a useful way to study gas phase photochemistry, and this could be the subject of future investigation.

Figure S3. Synthetic phosphorylated Abl biosensor reporter module. Added to K562 lysate followed by digestion and use as MRM calibration standard.

E:\Xcalibur\...\110811_02_pAbltide1 8/11/2011 12:15:30 PM RT: 0.00 - 10.58 NL: 4.84E5 450000 Channel A UV 110811_02 400000 _pAbltide1 350000 EAIpYAAPFAKKKbG 300000 ₹ 250000 200000 3.88 150000 7.09 5.09 6.30 6.97 100000 50000 3.57 7.61 7.81 8.28 8.74 9.48 9.95 0.34 0.84 1.03 1.55 2.82 0 2 5 6 10 3 8 Time (min) RT: 0.00 - 10.60 NL: 3.14E7 3.90 100 TIC MS 90 3.91 110811_02 _pAbltide1 3.89 80 3.92 Relative Abundance 3.93 60 50 40 5.10 5.41 5.50 30 3.85 4.92 20 5.88 7.09 7.14 7.15 4.82 4.53 10 4.46 2.69 3.31 3.80 7.45 8.39 9.26 9.56 10.24 2 3 6 8 9 10 Time (min) 110811_02_pAbItide1 #1151-1170 RT: 3.87-3.93 AV: 20 NL: 6.23E5 T: ITMS + p ESI Full ms [400.00-2000.00] 567.33 [M+3H]³⁺ 600000 [M+2H]²⁺ 550000 850.58 500000 450000 400000 350000 300000 250000 200000 150000 [M+H]+ 100000 486.25 50000 1133.17 1699.83 728.67 579.92 869.08 1000.67 1274.75 1359.33 1680.92 1701.83 1822.92 1455.83 1984.67 0 400 600 800 1000 1200 1400 1600 1800 2000

m/z

Figure S4. Additional Western blots from all replicates for data shown in Figure 2 in the main manuscript.



*A grey scale version of replicate 2 was shown in fig 2A.

Figure S5. Expanded view of MALDI-TOF spectrum for degradation products.

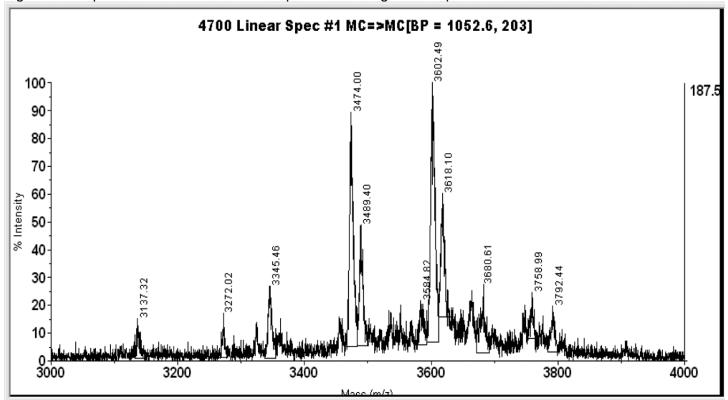
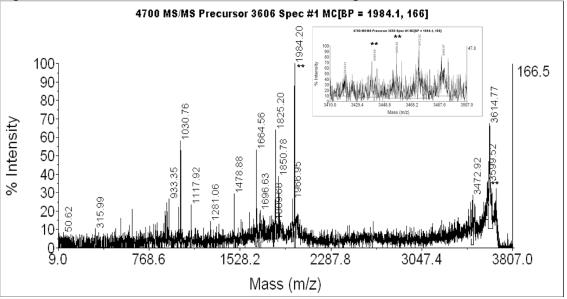


Figure S6. MALDI-TOF/TOF MS/MS for 3618/3602 fragment



y io			b		
n	sequence	m/z	ion	sequence	m/z
	EAIYAAPFAKKK _{biotin} GβNpaGCGGAP	3490.9		•	130.12
<mark>32</mark>	TYSPPPPPGGRKK-OH	<mark>6111</mark>	1	H2N-E	317
	AIYAAPFAKKK _{biotin} GβNpaGCGGAPT	<mark>3419.8</mark>			201.20
<mark>31</mark>	YSPPPPGGRKK-OH	<mark>8251</mark>	2	H2N-EA	177
	$IYAAPFAKKK_{biotin}G\beta NpaGCGGAPTY$	3306.7			314.36
30	SPPPPGGRKK-OH	2351	3	H2N-EAI	077
	$YAAPFAKKK_{biotin}G\beta NpaGCGGAPTY$	3143.5			477.53
29	SPPPPGGRKK-OH	4791	4	H2N-EAIY	637
	$AAPFAKKK_{biotin}G\beta NpaGCGGAPTYS$	3072.4			548.61
28	PPPPGGRKK-OH	6931	5	H2N-EAIYA	497
	$APFAKKK_{biotin}G\beta NpaGCGGAPTYSP$	3001.3			619.69
27	PPPPGGRKK-OH	9071	6	H2N-EAIYAA	357
	PFAKKK _{biotin} GβNpaGCGGAPTYSPP	2904.2	_		716.80
26	PPPGGRKK-OH	7431	7	H2N-EAIYAAP	997
25	FAKKK _{biotin} GβNpaGCGGAPTYSPPP	2757.0		LION FAIVAADE	863.98
25	PPGGRKK-OH	9811	8	H2N-EAIYAAPF	617
24	AKKK _{biotin} GβNpaGCGGAPTYSPPPP PGGRKK-OH	2686.0 1951	9	HON EAIVAADEA	935.06 477
24	KKK _{biotin} GβNpaGCGGAPTYSPPPPP	2557.8	9	H2N-EAIYAAPFA	1063.2
23	GGRKK-OH	4591	10	H2N-EAIYAAPFAK	3837
23	KK _{biotin} GβNpaGCGGAPTYSPPPPG	2429.6	10	HZN-LAHAAFI AN	1191.4
22	GRKK-OH	7231	11	H2N-EAIYAAPFAKK	1191.4
	K _{biotin} GβNpaGCGGAPTYSPPPPGG	2075.2	11	HZN-LAHAAH AKK	1545.8
21	RKK-OH	0231	12	H2N-EAIYAAPFAKKK _{biotin}	8197
	GβNpaGCGGAPTYSPPPPPGGRKK	2018.1		TIZIT ZITTO TITTO TO SOLUTI	1602.9
20	-OH** (observed m/z: 1984.20)	5051	13	H2N-EAIYAAPFAKKK _{biotin} G	3377
	bNpaGCGGAPTYSPPPPPGGRKK-	1825.0			1795.9
<mark>19</mark>	ОН	<mark>9051</mark>	14	H2N-EAIYAAPFAKKK _{biotin} GβNpa	9377
		<mark>1768.0</mark>			1853.0
<mark>18</mark>	GCGGAPTYSPPPPPGGRKK-OH	<mark>3871</mark>	15	H2N-EAIYAAPFAKKK _{biotin} GβNpaG	<mark>4557</mark>
		<mark>1664.9</mark>		H2N-	1956.1
<mark>17</mark>	CGGAPTYSPPPPPGGRKK-OH	<mark>0011</mark>	16	EAIYAAPFAKKK _{biotin} GβNpaGC	8417

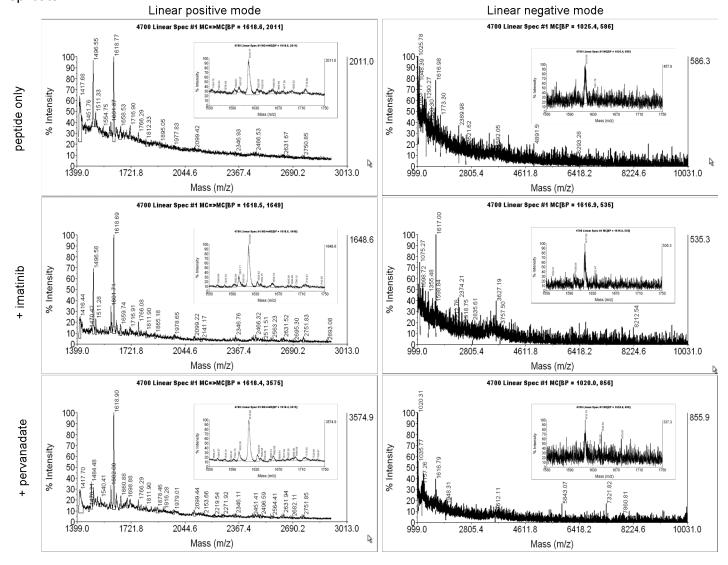
	16	COARTYCRRRRRR COLUMN	1607.8	47	H2N-	2013.2
-	16	GGAPTYSPPPPPGGRKK-OH	4831	17	EAIYAAPFAKKK _{biotin} GβNpaGCG	3597
	4-		1550.7	40	H2N-	2070.2
	15	GAPTYSPPPPPGGRKK-OH	9651	18	EAIYAAPFAKKK _{biotin} GβNpaGCGG	8777
					H2N-	
			<mark>1479.7</mark>		EAIYAAPFAKKK _{biotin} GβNpaGCGG	2141.3
	<mark>14</mark>	APTYSPPPPPGGRKK-OH	<mark>1791</mark>	19	A	6637
					H2N-	
			1382.6		EAIYAAPFAKKK _{biotin} GβNpaGCGG	2238.4
	13	PTYSPPPPPGGRKK-OH	0151	20	AP	8277
					H2N-	
			1281.4		EAIYAAPFAKKK _{biotin} GβNpaGCGG	2339.5
	12	TYSPPPPPGGRKK-OH	9671	21	APT	8757
					H2N-	
			1118.3		EAIYAAPFAKKK _{biotin} GβNpaGCGG	2502.7
	11	YSPPPPPGGRKK-OH	2111	22	APTY	6317
		1011111 COLUMN CIT			H2N-	3311
			1031.2		EAIYAAPFAKKK _{biotin} GβNpaGCGG	2589.8
	<mark>10</mark>	SPPPPPGGRKK-OH	4311	23	APTYS	4117
	10	SFFFF GGINN -OII	4311	23	H2N-	4117
			004.40			2000 0
	9	DDDDDCCDKK OH	934.12	24	EAIYAAPFAKKK _{biotin} GβNpaGCGG	2686.9
-	9	PPPPGGRKK-OH	<mark>671</mark>	24	APTYSP	5757
			007.04		H2N-	07040
	_	PPPP O PIKK OLL	837.01	0.5	EAIYAAPFAKKK _{biotin} GβNpaGCGG	2784.0
	8	PPPPGGRKK-OH	031	25	APTYSPP	7397
					H2N-	
	_		739.89		EAIYAAPFAKKK _{biotin} GβNpaGCGG	2881.1
	7	PPPGGRKK-OH	391	26	APTYSPPP	9037
					H2N-	
			642.77		EAIYAAPFAKKK _{biotin} GβNpaGCGG	2978.3
	6	PPGGRKK-OH	751	27	APTYSPPPP	0677
					H2N-	
			545.66		EAIYAAPFAKKK _{biotin} GβNpaGCGG	3075.4
L	5	PGGRKK-OH	111	28	APTYSPPPPP	2317
					H2N-	
			488.60		EAIYAAPFAKKK _{biotin} GβNpaGCGG	3132.4
	4	GGRKK-OH	931	29	APTYSPPPPPG	7497
					H2N-	
			431.55		EAIYAAPFAKKK _{biotin} GβNpaGCGG	3189.5
	3	GRKK-OH	751	30	APTYSPPPPGG	2677
H					H2N-	
			275.37		EAIYAAPFAKKK _{biotin} GβNpaGCGG	3345.7
	2	RKK-OH	051	31	APTYSPPPPGGR	1377
H		Tuux OII	001	01	H2N-	1011
			147.19		EAIYAAPFAKKK _{biotin} GβNpaGCGG	3473.8
	1	KK-OH	691	<mark>32</mark>		
- 1	ı	IVIV-OLI	l GO I	<u>5</u> 2	<u>APTYSPPPPPGGRK</u>	<mark>8737</mark>

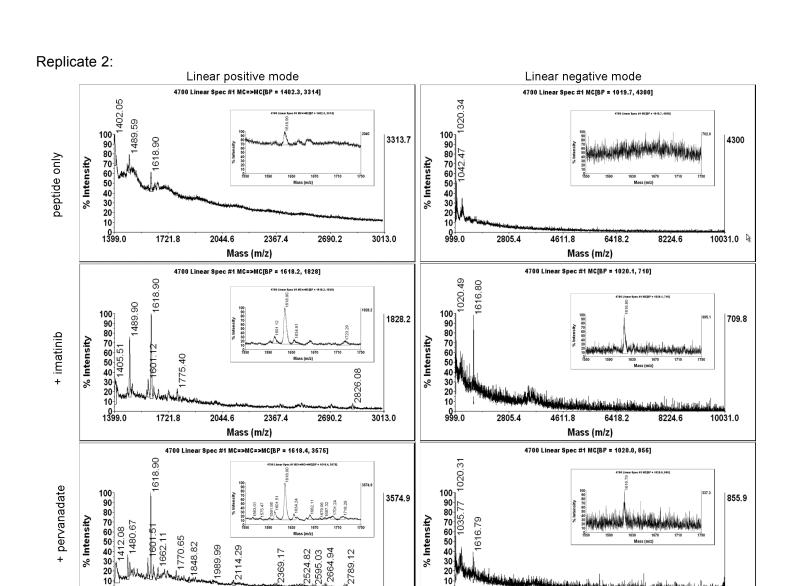
Predicted fragmentation for 3618/3602 degradation product

*Observed/tentatively assigned

^{**}lon observed (m/z 1984.2) has m/z consistent with MS/MS fragment y₂₀ exhibiting loss of 34 amu, which may predominate due to some effect of the proximity of the N-terminal glycine in this particular fragment (which, when protonated, could act as a proton donor); other potential photodegradation peaks also marked as ** in spectrum. For further discussion, see Fig. S2 and Scheme S1.

Figure S7. MALDI-TOF to analyze enriched reporter segment. Replicate 1:





% Intensity

2805.4

4611.8

Mass (m/z)

8224.6

10031.0

Table S8. Table of raw MRM data. See additional .csv file.

2044.6

% Intensity

40

10 0 1399.0

1721.8

Table S9. Table of peak integrations and calibration. See attached Excel file.

2367.4

Mass (m/z)

2524.82 2595.03 2664.94

2690.2

2789.12

3013.0