

CHEMBIOCHEM

Supporting Information

© Copyright Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, 2008

CHEM**BIO****CHEM**

Supporting Information

for

Photocontrollable Peptide-Based Switches Target the
Antiapoptotic Protein Bcl-xL

Sabine Kneissl, E. Joel Loveridge, Christopher Williams,
Matthew P. Crump, and Rudolf K. Allemann*

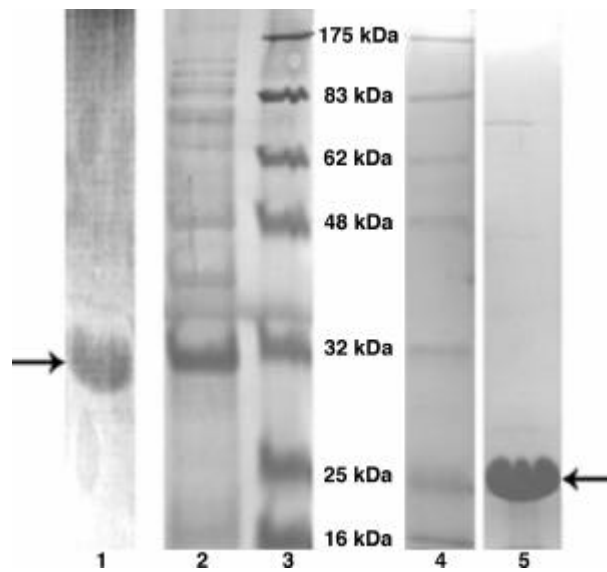


Figure S1. Polyacrylamide gel electrophoresis (under denaturing conditions) of purified Bcl-x_L after staining with Coomassie blue. Lanes are 1: purified Bcl-x_L; 2: crude Bcl-x_L; 3 and 4: marker; 5: purified ¹⁵N-labeled truncated-loop Bcl-x_L. The arrows indicate Bcl-x_L.

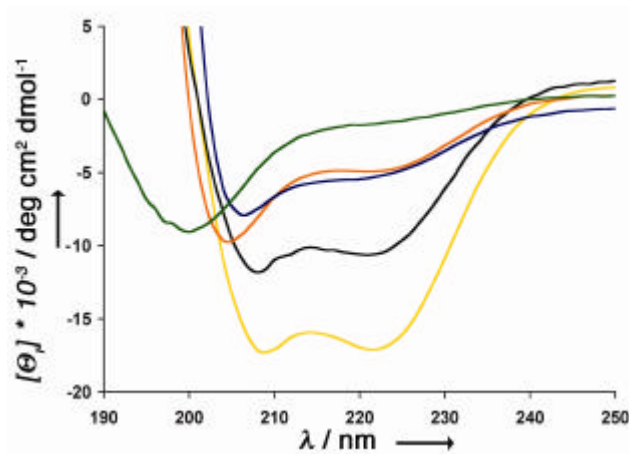


Figure S2. CD spectrum of Bid₉₁₋₁₁₁ⁱ⁺⁴ at 5 °C. Blue: dark adapted, orange: light induced, black: dark adapted in 20% TFE, and gold: light induced in 20% TFE. Trifluoroethanol was used as a cosolvent to induce more helicity in the peptides (similar to the expected effect on binding to Bcl-x_L) so that the structural difference upon photoswitching is more evident.

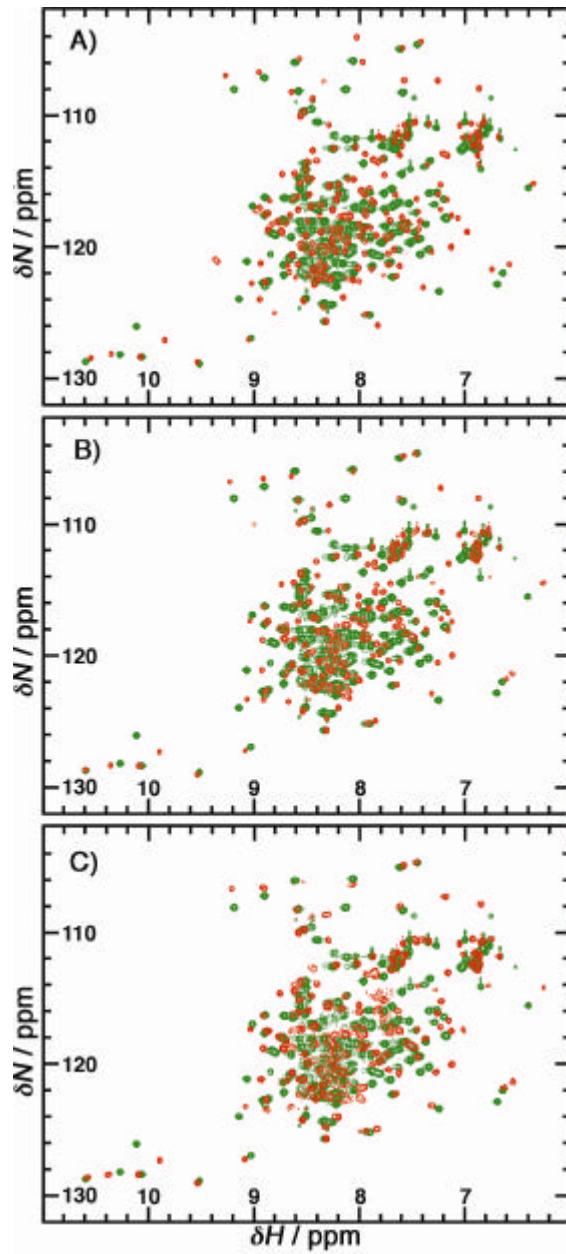


Figure S3. ^1H - ^{15}N HSQC NMR spectra of free Bcl- x_L 1-212 (Δ 45-84) (green) and in complex (red) with (A) wild type Bak₇₂₋₈₇, (B) dark-adapted alkylated Bak₇₂₋₈₇ⁱ⁺¹¹, and (C) dark-adapted alkylated Bak₇₂₋₈₇ⁱ⁺⁷.

Table S1. HPLC retention times and molecular weights of the peptides used in this study .

Peptide ^[a]	Sequence	RT (min)	m/z	Major MS peak	MW (g.mol ⁻¹)	Calc. MW (g.mol ⁻¹)
Bak ₇₂₋₈₇ ^{wildtype}	GQVGRQLAIGDDINR	31	[M+H] ⁺	1767	1766	1766
Bak ₇₂₋₈₇ ⁱ⁺⁷	GAVGRCLAIFGDCINR	40	[M+H] ⁺	1708	1707	1706
Bak ₇₂₋₈₇ ⁱ⁺⁷ X	GAVGRCLAIFGDCINR	34	[M+H] ⁺	2158	2157	2156
FAM Bak ₇₂₋₈₇ ⁱ⁺⁷	GAVGRCLAIFGDCINR	47	[M+H] ⁺	2024	2023	2023
FAM Bak ₇₂₋₈₇ ⁱ⁺⁷ X	GAVGRCLAIFGDCINR	40	[M-H] ⁻	2472	2473	2473
Bak ₇₂₋₈₇ ⁱ⁺¹¹	GCVGRALAAFGDCINR	38	[M+H] ⁺	1664	1663	1663
Bak ₇₂₋₈₇ ⁱ⁺¹¹ X	GCVGRALAAFGDCINR	42	[M+Na] ⁺	2139	2116	2114
FAM Bak ₇₂₋₈₇ ⁱ⁺¹¹	GCVGRALAAFGDCINR	42	[M+H] ⁺	1983	1982	1980
FAM Bak ₇₂₋₈₇ ⁱ⁺¹¹ X	GCVGRALAAFGDCINR	48	[M+H] ⁺	2430	2429	2430
Bid ₉₁₋₁₁₁ ⁱ⁺⁴	DIIRNIARHLACDVGCIDRSI	40	[M+H] ⁺	2395	2394	2394
Bid ₉₁₋₁₁₁ ⁱ⁺⁴ X	DIIRNIARHLACDVGCIDRSI	39	[M+H] ⁺	2846	2845	2844
FAM Bid ₉₁₋₁₁₁ ⁱ⁺⁴	DIIRNIARHLACDVGCIDRSI	49	[M+H] ⁺	2711	2710	2704
FAM Bid ₉₁₋₁₁₁ ⁱ⁺⁴ X	DIIRNIARHLACDVGCIDRSI	47	[M+H] ⁺	3149	3148	3154

[a] X: alkylated with azobenzene crosslinker; FAM: labeled with carboxyfluorescein at N-terminus

Table S2. Dissociation constants of the peptides used in this study with Bcl-x_L at 15 °C

K _D [nM]	Bak ₇₂₋₈₇ ⁱ⁺⁷	Bak ₇₂₋₈₇ ⁱ⁺¹¹	Bid ₉₁₋₁₁₁ ⁱ⁺⁴
Parent	134 ± 16	328 ± 19	117 ± 48
Dark adapted	825 ± 157	21 ± 1	1275 ± 139
Light induced	42 ± 9	48 ± 10	55 ± 4

Table S3. Dissociation constants of the peptides used in this study with HDM2 at 15 °C

K _D [nM]	Bak ₇₂₋₈₇ ⁱ⁺⁷	Bak ₇₂₋₈₇ ⁱ⁺¹¹	Bid ₉₁₋₁₁₁ ⁱ⁺⁴
Parent	n.d.	n.d.	n.d.
Dark adapted	>10 μM	>10 μM	>10 μM
Light induced	>10 μM	>10 μM	>10 μM

Table S4. Isotope-weighted chemical shift changes (ω) in the HSQC spectrum of Bcl-xL on addition of dark-adapted Bak₇₂₋₈₇ⁱ⁺¹¹. See text for details of how ω was calculated.

Residue	δ (ppm)		ω	ω/ω_{\max}
	Free Bcl-xL	Bcl-xL/Bak ₇₂₋₈₇ ⁱ⁺¹¹		
3Gln	8.42	8.44	0.056	0.059
	121.56	121.82		
5Asn	8.66	8.70	0.131	0.140
	120.92	121.55		
6Arg	8.06	8.07	0.205	0.218
	121.00	119.98		
7Glu	7.97	7.99	0.052	0.056
	117.17	117.42		
8Leu	7.80	7.87	0.116	0.124
	119.58	120.03		
9Val	7.75	7.62	0.139	0.148
	117.14	117.28		
10Val	8.34	8.40	0.114	0.121
	116.67	117.14		
11Asp	8.06	8.07	0.152	0.162
	121.00	120.24		
12Phe	8.32	8.23	0.166	0.177
	119.61	120.29		
13Leu	8.90	8.91	0.016	0.017
	116.22	116.27		
14Ser	8.51	8.55	0.051	0.054
	113.62	113.74		
15Tyr	8.26	8.31	0.082	0.087
	124.36	124.70		
17Leu	8.74	8.76	0.024	0.026
	117.94	117.85		
18Ser	8.39	8.43	0.041	0.043
	117.15	117.12		
19Gln	7.68	7.64	0.044	0.047
	122.24	122.17		
20Lys	7.26	7.26	0.052	0.056
	115.84	116.10		
21Gly	7.61	7.58	0.040	0.043
	104.97	104.84		

	7.89	7.83		
22Tyr	119.23	119.07	0.065	0.069
	8.57	8.62		
23Ser	114.76	114.44	0.088	0.094
	9.03	9.08		
24Trp	126.90	127.18	0.077	0.082
	10.07	10.10		
24Trp(Ne)	128.32	128.32	0.029	0.031
	8.24	8.29		
25Ser	111.53	110.51	0.210	0.224
	7.47	7.47		
26Gln	118.86	118.65	0.043	0.046
	7.34	7.21		
27Phe	115.91	115.14	0.196	0.210
	7.32	7.15		
28Ser	113.45	112.99	0.199	0.212
	8.37	8.27		
29Asp	122.11	122.39	0.111	0.119
	7.96	7.96		
30Val	118.68	118.53	0.030	0.032
	8.51	8.54		
31Glu	123.91	124.18	0.062	0.067
	8.44	8.46		
32Glu	121.56	122.07	0.103	0.110
	8.50	8.52		
33Asn	119.03	119.20	0.039	0.042
	8.33	8.35		
34Arg	121.48	121.56	0.028	0.030
	8.28	8.29		
35Thr	115.32	115.42	0.023	0.025
	8.40	8.41		
36Glu	122.52	122.61	0.021	0.022
	8.31	8.31		
37Ala	125.62	125.63	0.004	0.004
	8.63	8.64		
39Glu	120.51	120.65	0.028	0.030
	8.52	8.53		
40Gly	109.70	109.72	0.004	0.004
41Thr	8.25	8.21	0.043	0.045

	112.51	112.40		
	8.87	8.88		
42Glu	122.61	122.66	0.016	0.017
	8.49	8.46		
43Ser	114.88	114.77	0.040	0.043
	8.31	8.19		
44Glu	122.47	122.57	0.124	0.132
	8.22	8.18		
45Ala	118.21	118.51	0.068	0.073
	7.55	7.50		
46Val	120.28	120.53	0.073	0.078
	7.46	7.45		
47Lys	117.36	117.32	0.011	0.012
	8.17	8.17		
48Gln	115.82	115.72	0.020	0.021
	8.06	7.87		
49Ala	121.00	121.42	0.209	0.223
	8.51	8.48		
50Leu	120.04	119.81	0.052	0.056
	8.18	8.21		
51Arg	118.28	117.67	0.124	0.132
	7.91	8.00		
52Glu	115.87	116.15	0.108	0.115
	8.91	8.91		
53Ala	122.68	123.32	0.128	0.136
	9.19	9.23		
54Gly	108.04	106.79	0.252	0.269
	8.23	8.34		
55Asp	122.21	122.24	0.119	0.127
	8.27	8.27		
56Glu	120.30	119.96	0.068	0.073
	8.65	9.07		
57Phe	120.73	123.27	0.660	0.704
	8.15	8.40		
58Glu	116.19	113.92	0.517	0.551
	7.53	7.58		
59Leu	116.69	117.76	0.221	0.236
	8.04	8.06		
60Arg	117.00	117.16	0.037	0.039

	7.96	7.96		
61Tyr	118.68	118.42	0.051	0.055
	7.42	7.11		
62Arg	119.33	119.95	0.334	0.356
	8.55	8.69		
63Arg	118.61	117.62	0.239	0.255
	7.80	7.79		
64Ala	119.83	119.68	0.034	0.036
	8.56	7.70		
65Phe	115.49	114.47	0.885	0.944
	8.04	8.24		
66Ser	117.56	116.46	0.297	0.317
	8.38	8.47		
67Asp	120.47	120.99	0.140	0.150
	8.06	7.88		
68Leu	121.00	120.43	0.216	0.231
	8.45	9.00		
69Thr	109.51	109.97	0.551	0.588
	7.80	7.76		
70Ser	116.33	115.92	0.092	0.098
	7.92	7.75		
71Gln	118.67	118.42	0.177	0.189
	7.75	7.58		
72Leu	119.23	118.29	0.253	0.269
	8.26	8.22		
73His	119.14	119.38	0.064	0.068
	8.39	8.72		
74Ile	121.70	122.11	0.334	0.356
	7.91	7.84		
79Ala	125.13	124.92	0.077	0.082
	8.56	8.84		
80Tyr	119.80	120.53	0.320	0.341
	8.17	8.51		
81Gln	114.75	114.68	0.333	0.355
	7.63	7.38		
82Ser	112.35	113.91	0.401	0.427
	8.56	8.65		
84Glu	119.80	119.25	0.143	0.152
85Gln	7.59	7.62	0.295	0.315

	114.46	115.93		
	7.15	7.17		
86Val	118.01	117.89	0.027	0.029
	7.96	7.97		
87Val	118.68	119.17	0.099	0.106
	8.45	8.22		
88Asn	116.88	115.60	0.349	0.372
	7.37	7.36		
89Glu	118.26	118.00	0.053	0.057
	7.61	7.63		
90Leu	120.42	120.21	0.046	0.050
	7.03	6.86		
91Phe	112.65	108.03	0.938	1.000
	7.24	7.31		
92Arg	123.35	122.90	0.115	0.122
	8.44	8.71		
93Asp	118.13	116.27	0.460	0.491
	7.58	7.22		
94Gly	108.25	107.25	0.412	0.440
	8.27	8.27		
95Val	120.30	120.31	0.003	0.003
	6.39	6.25		
96Asn	115.49	114.45	0.250	0.267
	8.56	8.62		
97Trp	118.03	116.13	0.383	0.409
	10.27	10.37		
97Trp(Ne)	128.16	128.30	0.095	0.102
	8.61	8.64		
98Gly	105.98	106.39	0.090	0.096
	8.23	8.12		
99Arg	121.11	121.49	0.132	0.141
	7.61	7.81		
100Ile	120.42	120.68	0.202	0.215
	8.38	8.35		
101Val	120.47	120.73	0.060	0.064
	7.71	7.72		
102Ala	121.13	120.38	0.151	0.161
	8.11	8.23		
103Phe	119.09	119.53	0.145	0.154

	8.06	8.06		
104Phe	121.00	120.04	0.192	0.204
	8.51	8.41		
105Ser	114.16	112.84	0.283	0.301
	9.14	8.67		
106Phe	123.93	123.48	0.482	0.514
	8.06	8.05		
107Gly	105.86	106.01	0.029	0.031
	8.90	8.91		
108Gly	107.14	106.54	0.121	0.129
	8.34	8.20		
109Ala	124.30	123.94	0.157	0.167
	8.31	8.38		
110Leu	118.68	118.12	0.133	0.142
	8.22	8.22		
111Cys	118.81	118.68	0.027	0.029
	8.22	8.10		
112Val	118.18	118.43	0.130	0.139
	8.18	8.25		
113Glu	118.82	118.32	0.122	0.130
	7.77	7.84		
114Ser	113.28	113.24	0.077	0.082
	7.42	7.25		
115Val	120.57	120.53	0.167	0.178
	8.39	8.36		
116Asp	121.70	122.46	0.156	0.166
	7.69	7.84		
117Lys	115.57	115.17	0.175	0.186
	7.96	7.85		
118Glu	113.64	112.92	0.180	0.192
	8.53	8.68		
119Met	118.71	118.25	0.178	0.190
	8.80	8.92		
120Gln	119.09	118.79	0.138	0.147
	7.96	7.94		
121Val	116.68	117.67	0.200	0.213
	7.89	8.00		
122Leu	116.86	115.46	0.303	0.324
123Val	7.61	7.45	0.257	0.275

	120.42	119.41		
	8.40	8.57		
124Ser	110.51	109.91	0.212	0.226
	6.69	6.54		
125Arg	122.80	121.34	0.328	0.350
	8.12	8.12		
126Ile	118.12	117.66	0.091	0.098
	7.75	7.69		
127Ala	119.23	118.98	0.080	0.085
	7.61	7.34		
128Ala	120.42	120.16	0.281	0.300
	9.07	8.92		
129Trp	121.08	121.06	0.143	0.152
	10.12	9.90		
129Trp(Ne)	126.02	127.27	0.332	0.354
	9.01	9.04		
130Met	116.88	117.32	0.093	0.099
	8.27	8.28		
131Ala	120.30	120.16	0.031	0.034
	8.63	8.74		
132Thr	116.37	114.56	0.377	0.402
	8.50	8.90		
133Tyr	123.38	122.96	0.410	0.437
	8.83	8.71		
134Leu	118.92	118.71	0.124	0.133
	8.43	8.48		
135Asn	116.96	115.88	0.221	0.236
	8.56	8.55		
136Asp	115.27	115.03	0.048	0.051
	8.12	7.92		
137His	111.82	113.04	0.316	0.337
	7.29	7.22		
138Leu	116.75	117.16	0.106	0.113
	8.72	8.68		
139Glu	121.36	121.18	0.056	0.060
	7.19	7.12		
141Trp	117.69	117.40	0.089	0.095
	9.52	9.54		
141Trp(Ne)	128.84	129.00	0.040	0.043

	8.52	8.56		
142Ile	122.08	122.18	0.044	0.047
	7.62	7.54		
144Glu	118.82	118.29	0.131	0.140
	7.20	7.15		
145Asn	116.36	116.71	0.082	0.087
	7.45	7.46		
146Gly	104.61	104.65	0.020	0.021
	8.58	8.58		
147Gly	108.16	108.21	0.009	0.010
	8.71	8.70		
148Trp	117.95	118.01	0.016	0.017
	10.60	10.61		
148Trp(Ne)	128.67	128.63	0.010	0.011
	8.90	8.87		
149Asp	117.59	117.41	0.043	0.046
	7.62	7.58		
150Thr	116.58	116.43	0.055	0.059
	6.63	6.60		
151Phe	121.96	121.76	0.055	0.059
	7.89	7.78		
152Val	117.03	116.40	0.162	0.173
	7.51	7.43		
153Glu	119.68	119.38	0.098	0.105
	7.68	7.65		
154Leu	116.88	117.77	0.180	0.192
	8.17	8.20		
155Tyr	114.75	115.25	0.103	0.110
	8.13	8.28		
156Gly	108.04	108.53	0.183	0.195
	8.45	8.58		
157Asn	117.99	117.36	0.180	0.192
	8.45	8.61		
158Asn	118.68	118.73	0.160	0.171
	8.22	8.15		
159Ala	122.96	122.97	0.068	0.073
	8.16	8.27		
160Ala	121.24	121.09	0.118	0.126
161Ala	8.05	8.00	0.053	0.057

	121.93	121.84		
	8.33	8.31		
162Glu	118.02	118.12	0.032	0.035
	8.12	8.14		
163Ser	114.73	114.85	0.031	0.033
	8.05	8.04		
164Arg	121.37	121.59	0.046	0.049
	8.11	8.14		
165Lys	120.51	120.71	0.053	0.057
	8.44	8.45		
166Gly	108.85	108.92	0.021	0.022
	8.14	8.14		
167Gln	119.14	119.19	0.010	0.010
	8.53	8.55		
168Glu	121.03	121.15	0.030	0.032
	8.28	8.29		
169Arg	120.56	120.82	0.053	0.056
	8.19	8.10		
170Leu	122.27	122.67	0.126	0.134