Supporting Information

In culture cross-linking of bacterial cells reveals large scale dynamic protein-protein interactions at the peptide level

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Table S1. Proteins identified in SCX fractions 1-12 (separate xlsx file).

Table S2. Inter-protein cross-linked peptides (separate xlsx file).

Table S3. Intra-protein cross-linked peptides (cross-links with different sequences from the same protein) (separate xlsx file)

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Table S4

Supplementary Table 4. Effect of variations of assignment criteria on the number of identified cross-linked peptides (spectral counts) and the false discovery rate (FDR)

XL type	criteria			spectral counts		FDR	
	score	*minimum nr of assigned y ions to peptides ≤ 10 amino acids*	*minimum nr of assigned y ions to peptides > 10 amino acids*	*unique*	*total*	*unique*	*total*
intra protein	≥ 40	3	4	295	1273	0%	0%
decoy intra protein	≥ 40	3	4	0	0		
intraprotein	≥ 40	2	3	343	1614	0%	0%
decoy intra protein	≥ 40	2	3	0	0		
intraprotein	≥ 40	1	1	369	1920	0%	0%
decoy intra protein	≥ 40	1	1	0	0		
intra protein	≥ 25	1	1	370	1990	0%	0%
decoy intra protein	≥ 25	1	1	0	0		
interprotein (different peptides)	≥ 40	3	4	58	231	0%	0%
decoy interprotein	≥ 40	3	4	0	0		
interprotein	≥ 25	3	4	59	236	2%	0.4%
decoy interprotein	≥ 25	3	4	1	1		
interprotein	≥ 40	2	3	80	285	14%	4%
decoy interprotein	≥ 40	2	3	13	13		
interprotein same peptides (homodimers)	≥ 40	3	4	24	67	0%	0%
decoy homodimers	≥ 40	3	4	0	0		
homodimers	≥ 40	2	3	24	68	0%	0%
decoy homodimers	≥ 40	2	3	0	0		
homodimers	≥ 25	3	4	25	76	0%	0%
decoy homodimers	≥ 25	3	4	0	0		

Table S5. Distance measurements for HADDOCK output data on the combined *in vitro* and *in vivo* cross-linking data for the interaction of δ with the β' subunit of RNAP.

		Distance Å (Cα-Cα)				
Cluster	Cluster model	K208	K1104	K1152	Total	Rank
1	1	15.8	34.4	25.1	75.3	3
	2				0	
	3				0	
	4				0	
2	1	20.5	35.5	28.7	84.7	10
	2				0	
	3				0	
	4				0	
3	1	17.2	34.5	24.5	76.2	4
	2				0	
	3				0	
	4				0	
4	1	10.4	29.9	24.3	64.6	1
	2	10.2	29.3	23.9	63.4	
	3	10.8	29.7	23.8	64.3	
	4	10.6	30.1	24.2	64.9	
5	1	17	34.5	25.4	76.9	6
	2				0	
	3				0	
	4				0	
6	1	11.4	34.2	24.3	69.9	2
	2	11.4	34.5	24.1	70	
	3	10.6	33.7	23.6	67.9	
	4	11	34.3	24	69.3	
7	1	19.2	34.1	27.9	81.2	7
	2				0	
	3				0	
	4				0	
9	1	20.4	29	32	81.4	8
	2				0	
	3				0	
	4				0	
10	1	19.3	35.9	27.7	82.9	9
	2				0	
	3				0	
	4				0	
13	1	16.8	33.8	26	76.6	5
	2				0	
	3				0	
	4				0	

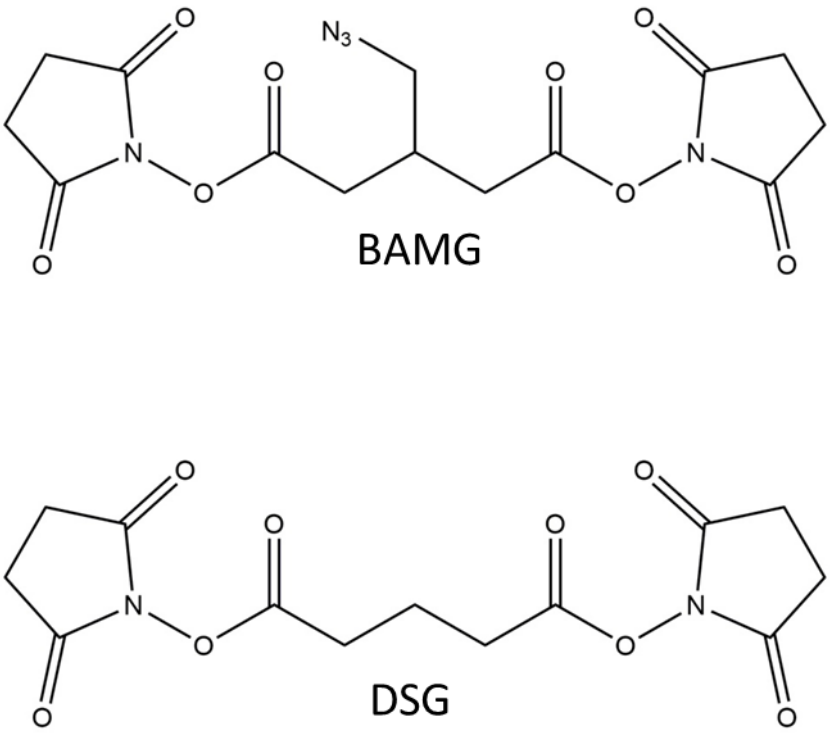

Figure S1. Structures of cross-linkers used in this study. BAMG, bis(succinimidyl)-3-azidomethyl-glutarate; DSG, disuccinimidyl-glutarate.

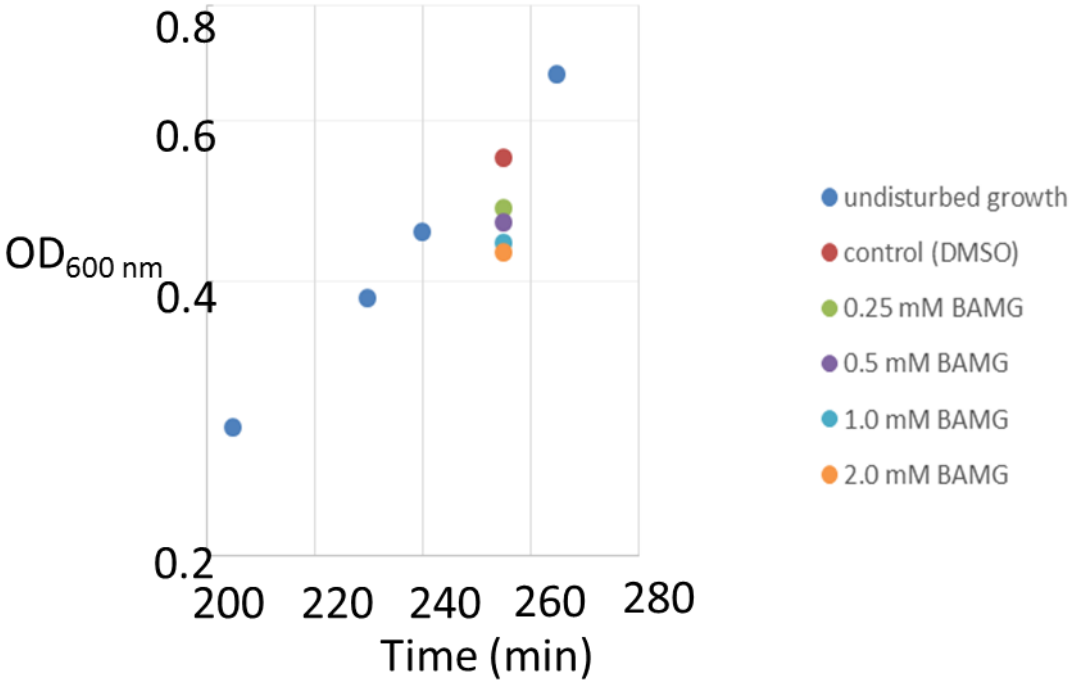

Figure S2. Growth arrest of *Bacillus subtilis* after addition of various concentrations of BAMG. Six culture flasks with 10 ml pre-warmed minimal medium containing 1.2 mM glutamine were inoculated with 0.4 ml from an exponentially growing pre-culture to obtain a final $OD_{600\ nm}$ = 0.012. Growth was followed in one culture flask. At t = 240 min, 50 µl of a DMSO solution containing the required amount of BAMG was rapidly added to the each of the five other cultures. After 5 min the reaction was quenched by addition of 0.5 ml 1 M Tris-HCL pH 8.0 and 10 min later the $OD_{600\ nm}$ was measured. Values were corrected for volume changes by the additions of cross-linker and quenching solutions. Extracts of cross-linked samples were subjected to SDS-PAGE analysis before and after digestion with trypsin (See Figure S3).

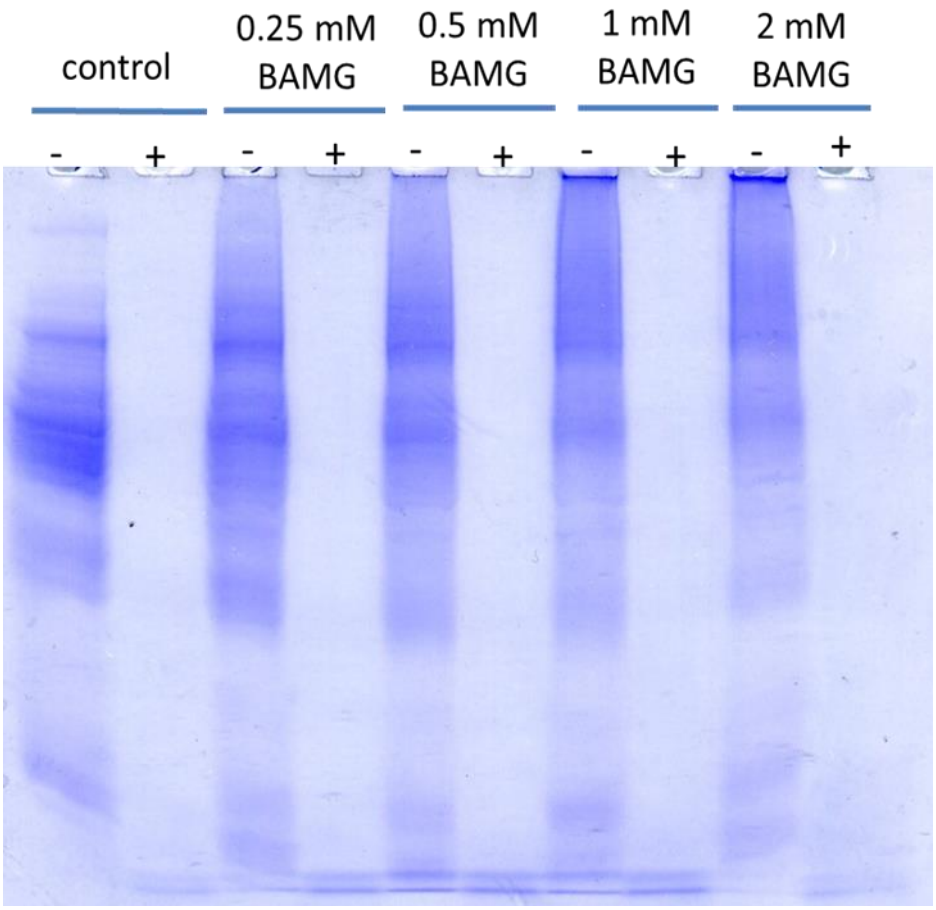

Figure S3. Extracted proteins after in vivo cross-linking with different concentrations BAMG of exponentially growing *B. subtilis* can be digested efficiently. Proteins before and after digestion were concentrated by centrifugation on a 10 kDa cut-off filter before SDS-PAGE analysis on 12% gels. -, before digestion with trypsin; +, after digestion with trypsin. Gels were stained with Coomassie Briliant Blue.

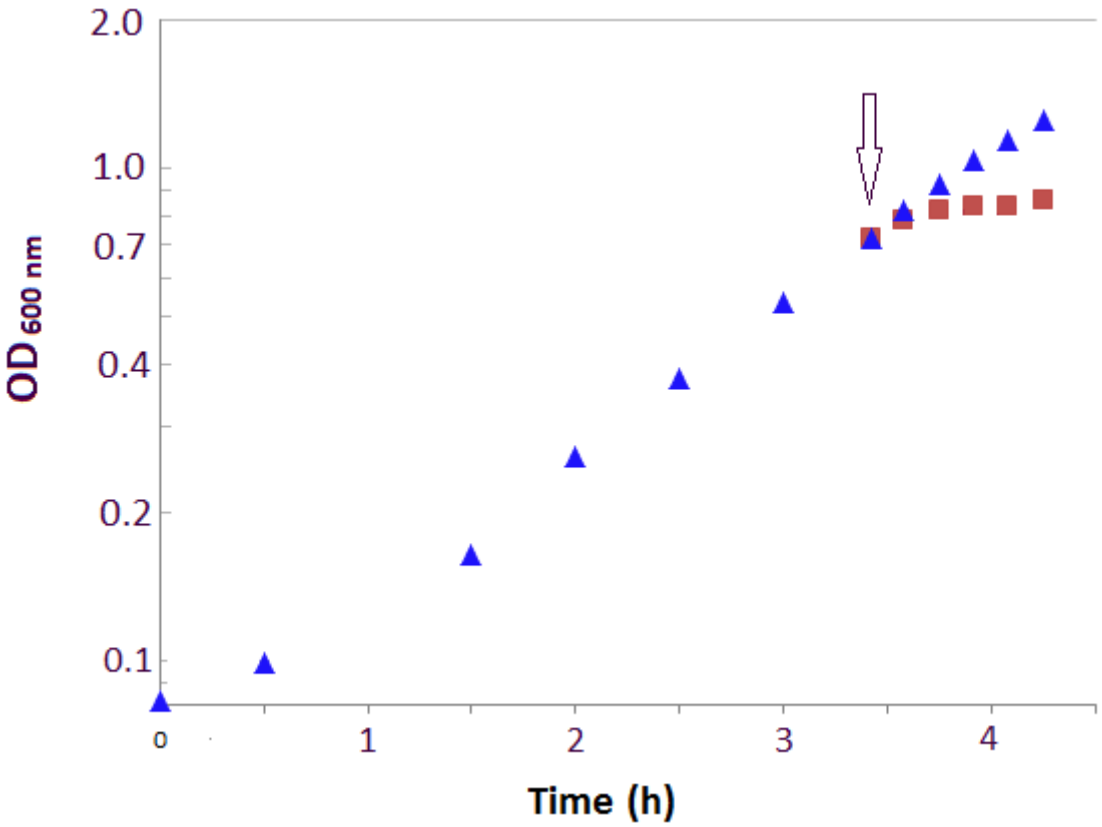

Figure S4. Growth curve of E coli in MOPS medium supplemented with 0.16% N-acetylglucosamine and 0.1 mM NH_4Cl. Blue triangels, undisturbed growth; arrow, addition of 2 mM BAMG or DMSO (control). Brown squares, growth after addition of BAMG.

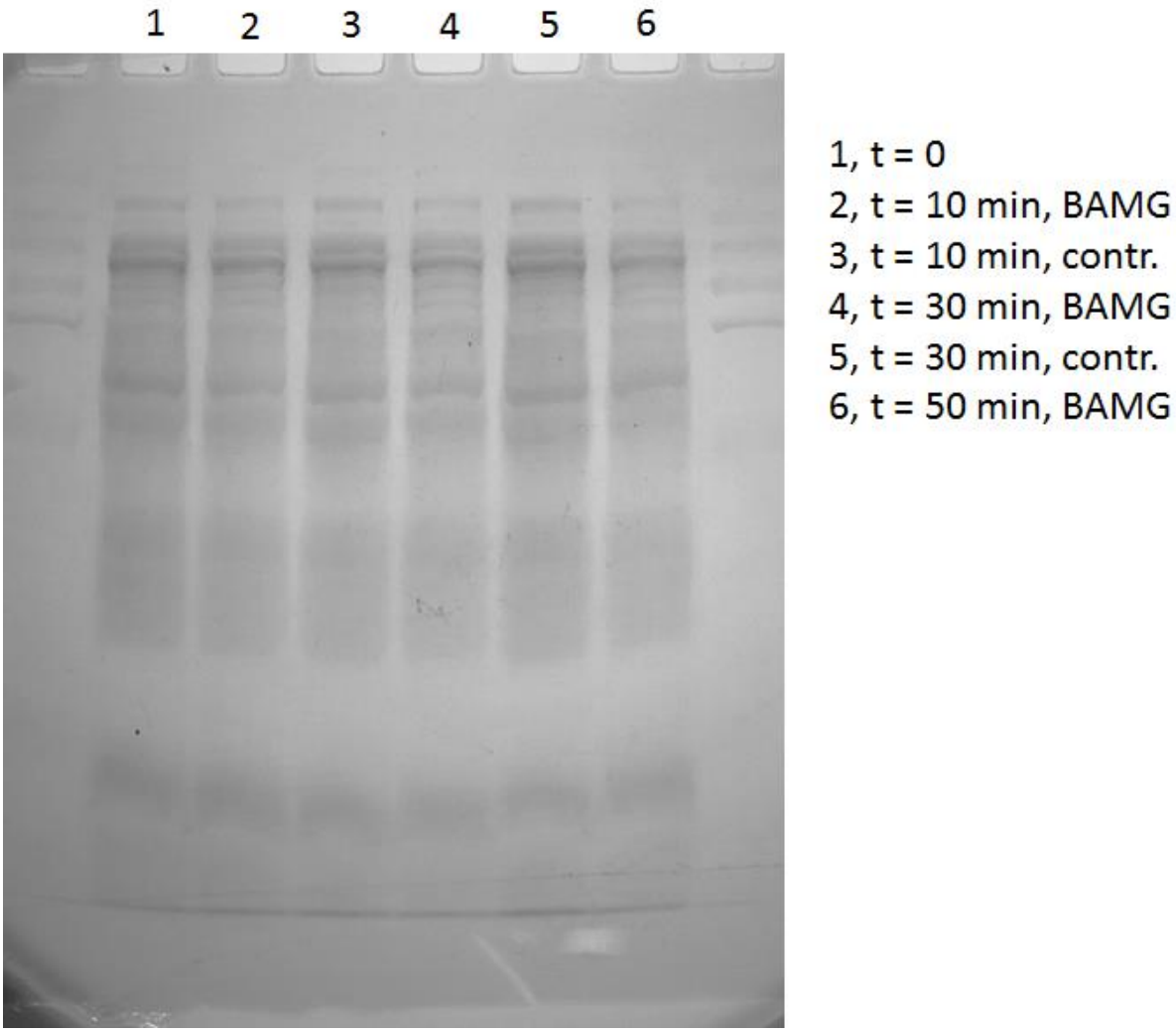

Figure S5. Coomassie Briliant Blue stained SDS-PAGE of extracted proteins from exponentially growing *E. coli* before and after addition of 2mM of the cross-linker BAMG directly in the growth medium. The similarity of the protein band patterns in the different lanes indicates that no substantial cross-linking had occurred, in contrast with in vivo cross-linking of exponentially growing *B. subtilis*.

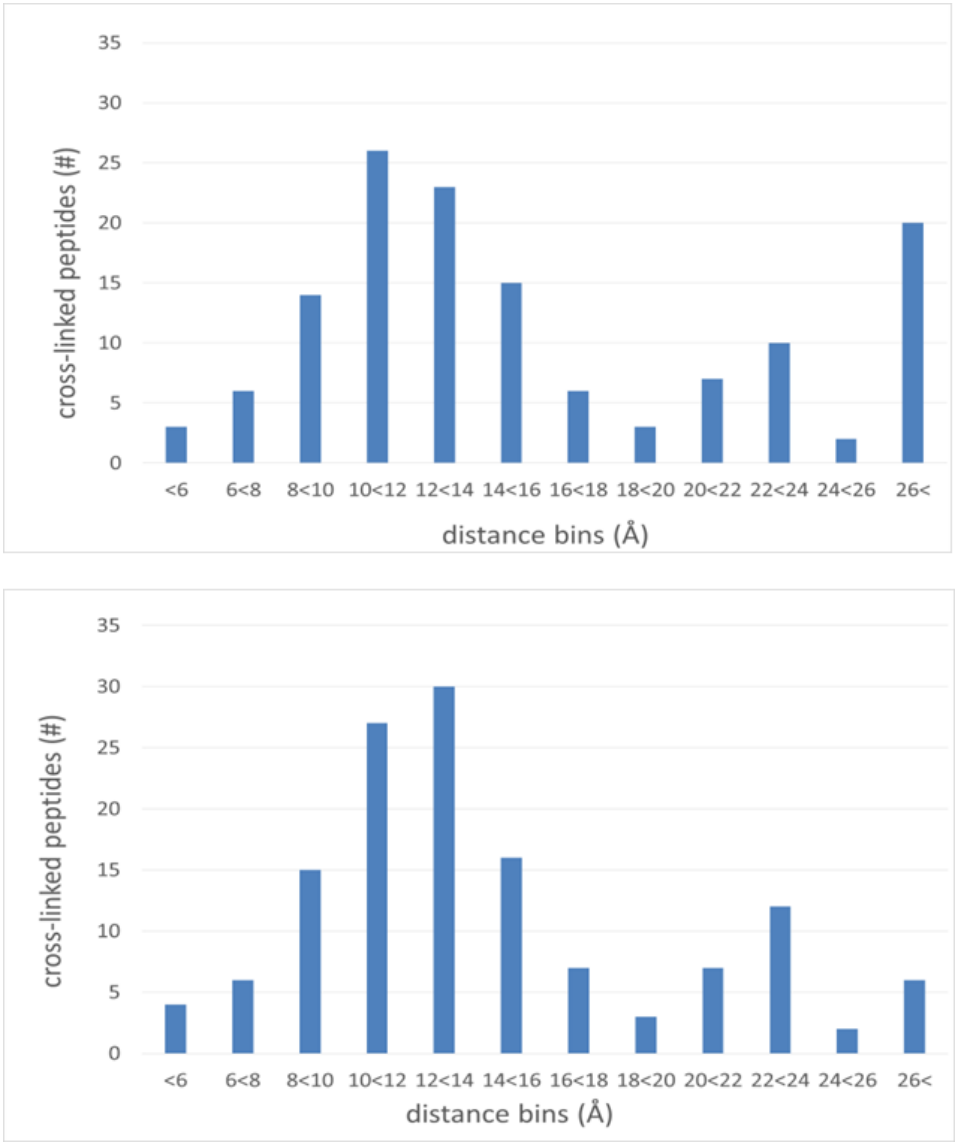

Figure S6. Distribution of 135 cross-link distances derived from 31 crystal structures comprising 43 different proteins with non-overlapping cross-linked peptide sequences from the same protein sequence, denoted intra-protein cross-links. The dataset was obtained from 12 crystal structures comprising 20 *B. subtilis* proteins and 19 crystal structures from homologous proteins, selected for 23 other *B. subtilis* proteins with at least three cross-linked peptides (**Table S3**). Upper panel, distribution of distances assuming only intra-protein cross-linked peptides. The majority (85,2%,) of the cross-links is within the 25.7 Å limit of BAMG. Lower panel, distribution of distances upon mapping cross-links exceeding the 25.7 Å between identical proteins in the crystal structures (inter-protein cross-links). This was the case with 14 cross-links, raising the percentage of species with distances < 25.7 Å to 95.6%.