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SUPPLEMENTARY INFORMATION

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A cross-linking/mass spectrometry workflow based on MS-cleavable cross-linkers and the MeroX software for studying protein structures and protein–protein interactions

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Supporting Information

A Cross-linking/Mass Spectrometry Workflow Based on MS-Cleavable Cross-Linkers and the MeroX Software for Studying Protein Structures and Protein-Protein Interactions

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Functional description of MeroX

1. *Peptide database generation:*

    a. Amino acid sequences of proteins are imported from standard fasta files. Static modifications are applied by changing the character of modified amino acids in the sequence string of all proteins, e.g. for cysteine alkylation ‘C’ is changed to ‘B’ at all positions).

    b. All sequences in the database are shuffled, while keeping the proteolysis sites at the same position to generate a decoy database. For reproducibility, the shuffling is seeded with each protein sequence. All peptides that are generated from the decoy database are flagged as decoy and added to the peptide database.

    c. In-silico proteolysis: All possible peptides originating from all proteins are generated based on the proteolysis sites and missed cleavage information given in the settings file. The peptides are stored alongside with the positional information (protein ID and location on the primary sequence).

    d. Duplicate entries (peptides with identical amino acid sequences) are combined by adding the positional information (see c).

    e. Variable modifications are applied to all peptides by changing the character of the modified amino acid residues in all combinations up to the user-defined maximum number of modifications per peptide, e.g. ‘M’ is changed to ‘m’ for methionine oxidation.

2. *MS data analysis (description for RISE mode):*

    a. Mass spectrometric data are imported from different file types (MGF, mzXML, mzML, pkl) spectrum-by-spectrum in a loop over all spectra in a single file.

        i. A single spectrum is processed to remove the noise by splitting the spectrum into < 11 bins of < 51 signals. The noise for each bin is iteratively calculated until the user-defined signal-to-noise ratio is reached.

        ii. The spectrum is deisotoped and charge information is stored for each signal.

        iii. The spectrum is deconvoluted. For signals with unknown charge states, all possible charge states are assumed. Resulting masses are sorted according to their masses.

        iv. The list of masses is analyzed for matching cross-linker fragment ion pairs. Masses of matching peptides are calculated from the fragment ion pairs.

        v. Identified mass pairs are compared to the generated peptide database.

vi. All combinations of filtered peptides matching the precursor mass with the user-defined precision are scored as cross-link candidates.

vii. Scoring is performed by comparing the theoretical fragment ions of a cross-link candidate to the spectrum and four spectra with slightly shifted masses. For each comparison, four features are evaluated: (I) number of identified signals per spectrum size $\left(\frac{H}{S}\right)$, (II) number of identified signals per possible fragment ions of the cross-link candidate $\left(\frac{H}{N}\right)$, (III) presence and intensity of the cross-linker fragment ions, and (IV) number and length of peptide backbone ion series. For each feature, the numerical difference $f_{n,0}$ of the original spectrum compared to the average $\bar{f_n}$ of the shifted spectra and the standard deviation σ_n is calculated:

$$x_n = f_{n,0} - (\bar{f_n} + \sigma_n) \text{ (eq. 1)}$$

viii. From implemented model distributions, the probability of a feature (P_n) being a random match is calculated from x_n (eq. 1) and are combined to a score (s):

$$s = -15 \cdot \log\left(P_{\frac{H}{S}} \cdot P_{\frac{H}{N}} \cdot P_{series} \cdot P_{reporter}\right) \text{ (eq. 2)}$$

ix. Scoring is performed for all combinations of cross-linking sites.

x. The probability for each cross-linking site combination is calculated by a pairwise comparison matrix. The comparison is solely based on the intensity of branched fragment ions that differ between the two cross-link sites. Branched ions are defined as peptide backbone fragment ions containing parts of the linker or the linker with the second peptide. The cross-linking site with the highest probability is reported in the result list.

xi. Cross-linking candidates exceeding the user-defined score minimum are stored to the result file alongside the analyzed spectrum.

xii. For FDR estimation, the numerical score values are separately stored for target candidates (both peptides from the target database) and for decoy candidates (at least one peptide from the decoy database).

3. FDR estimation is performed by first sorting all numerical scores obtained from target and decoy candidates. FDR values for a certain score cut-off are calculated by counting all decoy and target candidates above the threshold using:

$$FDR = \frac{N_{decoy}}{3 \cdot N_{target}} \text{ (eq. 3)}$$

4. The data are filtered based on the user-defined FDR cut-off in the result file.

MeroX data formats

MeroX result files (*.zhrm) are zip files containing all relevant information of a single analysis. All spectra are stored in a binary coded format with a UUID for referencing. The result table is included as ‘result.csv’, the applied settings are stored in the MeroX-specific format as ‘properties.mxf’. The amino acid sequence length of all protein entries is stored for xVis compatibility and saved as ‘proteinLength.csv’. The numerical score values of all decoy and target hits are stored in ‘decoyBase.csv’, general information on the analysis is stored in report.txt.

The MeroX settings files (*.mxf) are text files. Each setting is saved in a separate line starting with the name of the setting in capital letters followed by ‘=’ and the corresponding value (e.g. ‘MINMASS=200’). Comments can be added after a double slash //. The settings file contains several blocks (monoisotopic masses of elements, amino acids, static modifications, variable modifications, proteases, ion types, and cross-linker) where specific information is combined. The majority of settings can be modified from within the graphical user interface.

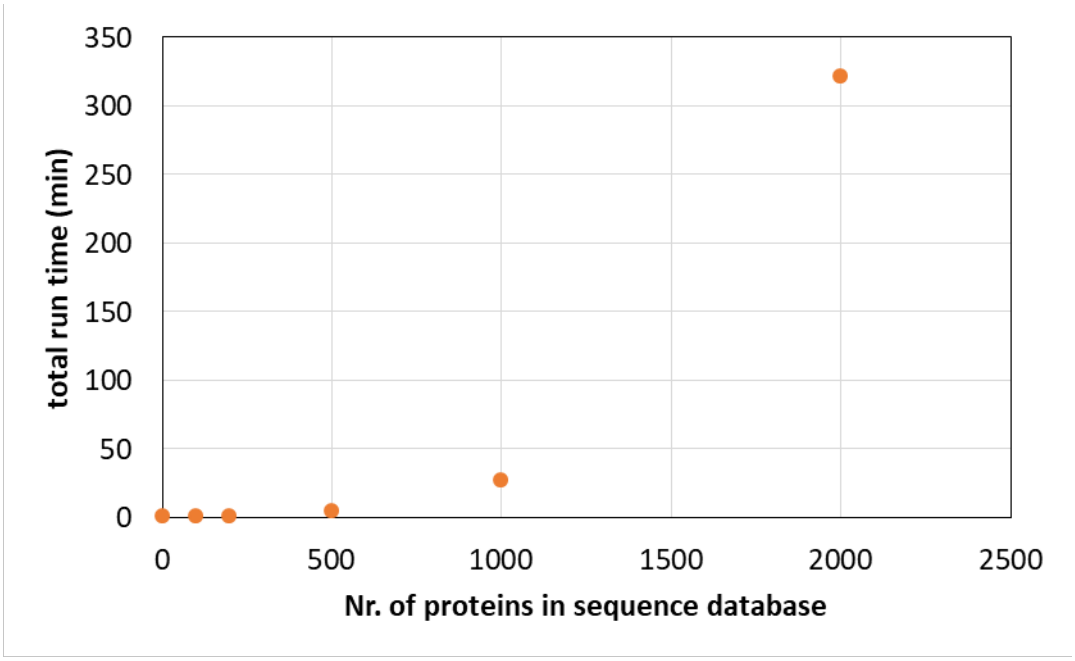

Figure S1: Correlation of database size and calculation time for the MeroX 1.6.6 software. For database containing less than 1000 proteins, runtimes are in the range of minutes. For larger databases the runtime increases. We recommend using less than 2000 proteins in the sequence database. Analyses were done on a personal computer with Intel core i5 processor (4 parallel threads) and 8 GB RAM allocated to the java runtime environment.

Cross-linking of BSA

Experimental procedure

10 µM BSA (in 20 mM HEPES, pH 7.5) was cross-linked with 1 mM DSBU at room temperature for 60 min. The cross-linking reaction was quenched by adding TRIS buffer to a final concentration of 20 mM. *In-solution* digestion was conducted with trypsin using an enzyme-protein ratio of 1:50 at 37 °C overnight. The resulting peptide mixture was applied to LC/MS/MS analysis (Ultimate 3000 RSLC nano-HPLC system coupled to an Orbitrap Fusion Tribrid mass spectrometer, both Thermo Fisher Scientific), applying stepped HCD (normalized collision energies 27 %, 30 % and 33 %).

The experiment was conducted in three replicates, i.e., three independent cross-linking reactions were performed in parallel.

```
 25     DTHKSE IAHRFKDLGE EHFKGLVLIA FSQYLQQCPF DEHVKLVNEL
 71 TEFAKTCVAD ESHAGCEKSL HTLFGDELCK VASLRETYGD MADCCEKQEP
121 ERNECFLSHK DDSPDLPKLK PDPNTLCDEF KADEKKFWGK YLYEIARRHP
171 YFYAPELLYY ANKYNGVFQE CCQAEDKGAC LLPKIETMRE KVLTSSARQR
221 LRCASIQKFG ERALKAWSVA RLSQKFPKAE FVEVTKLVTD LTKVHKECCH
271 GDLLECADDR ADLAKYICDN QDTISSKLKE CCDKPLLEKS HCIAEVEKDA
321 IPENLPPLTA DFAEDKDVCK NYQEAKDAFL GSFLYEYSRR HPEYAVSVLL
371 RLAKEYEATL EECCAKDDPH ACYSTVFDKL KHLVDEPQNL IKQNCDQFEK
421 LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS RSLGKVGTRC CTKPESERMP
471 CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC TESLVNRRPC FSALTPDETY
521 VPKAFDEKLF TFHADICTLP DTEKQIKKQT ALVELLKHKP KATEEQLKTV
571 MENFVAFVDK CCAADDKEAC FAVEGPKLVV STQTALA
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Figure S2: Amino acids sequence of BSA without signal peptide.

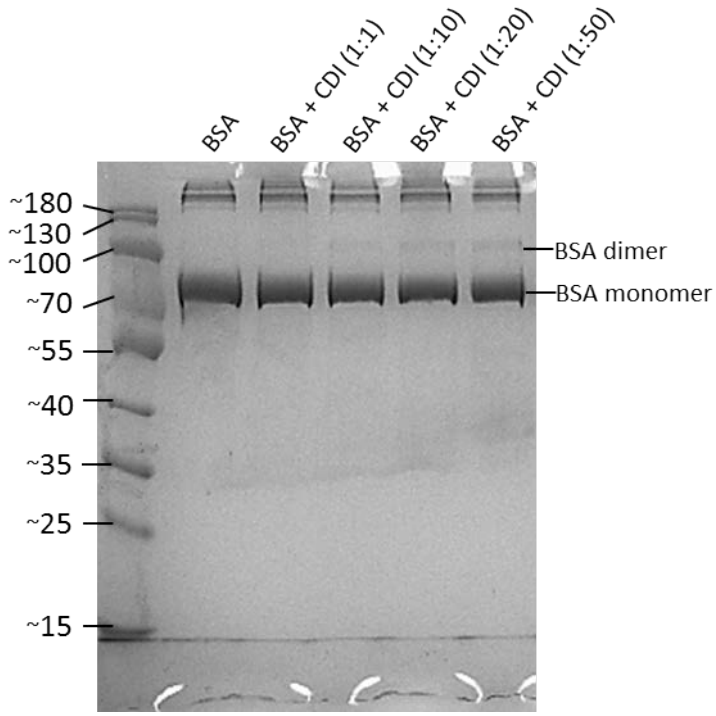

Figure S3: Cross-linking reaction of BSA with different molar excess of CDI cross-linker. Formation of the BSA dimer on the gel is observed starting at a 10-fold molar excess of CDI over BSA.

Table S1: Unique BSA cross-links identified for different CDI concentrations.

10 µM BSA, 20 mM HEPES, pH 8, 20 °C															
50-fold molar excess of CDI				20-fold molar excess of CDI				10-fold molar excess of CDI				Equimolar concentration of CDI			
Entry	Site (1)	Site (2)	Cα-Cα (Å)	Entry	Site (1)	Site (2)	Cα-Cα (Å)	Entry	Site (1)	Site (2)	Cα-Cα (Å)	Entry	Site (1)	Site (2)	Cα-Cα (Å)
1	K187	Y156	9.3	1	K187	Y156	9.3	1	K187	Y156	9.3	1	K431	Y451	9.5
2	K413	Y410/T411	4.7/5.4	2	K413	Y410/T411	4.7/5.4	2	K413	Y410/T411	4.7/5.4	2	K187	Y156	9.3
3	K524	K520	6.5	3	K524	K520	6.5	3	K524	K520	6.5	3	K524	Y400	11.1
4	K377	K350	13.7	4	K377	K350	13.7	4	K377	K350	13.7	4	K524	K520	6.5
5	Y262	K232	9.1	5	K524	K544	13.4	5	Y262	K232	9.1				
6	Y84	K465	16.1	6	Y262	K232	9.1	6	K187	Y160	11.6				
7	K204	K350	17	7	K204	K350	17	7	K187	Y451	12.7				
8	Y340	K377	12.1	8	Y340	K377	12.1	8	K524	Y400	11.1				
9	K211	K350	13.6	9	K187	Y160	11.6	9	K431	Y451	9.5				
10	K187	Y160	11.6	10	K187	Y451	12.7	10	K187	K180	11				
11	K187	Y451	12.7	11	K524	Y400	11.1	11	K187	K439	17.8				
12	K4	T52	13.5	12	K187	K116	17.4								
13	K524	Y400	11.1	13	K431	Y451	9.5								
14	K431	Y451	9.5	14	K187	K180	11								
15	K187	K180	11	15	K187	K439	17.8								
16	K187	K439	17.8	16	K431	K439	13.5								
17	K211	K239	9.6												
18	K431	K439	13.5												
19	D1(N-ter)/T2	K12	Not resolved												

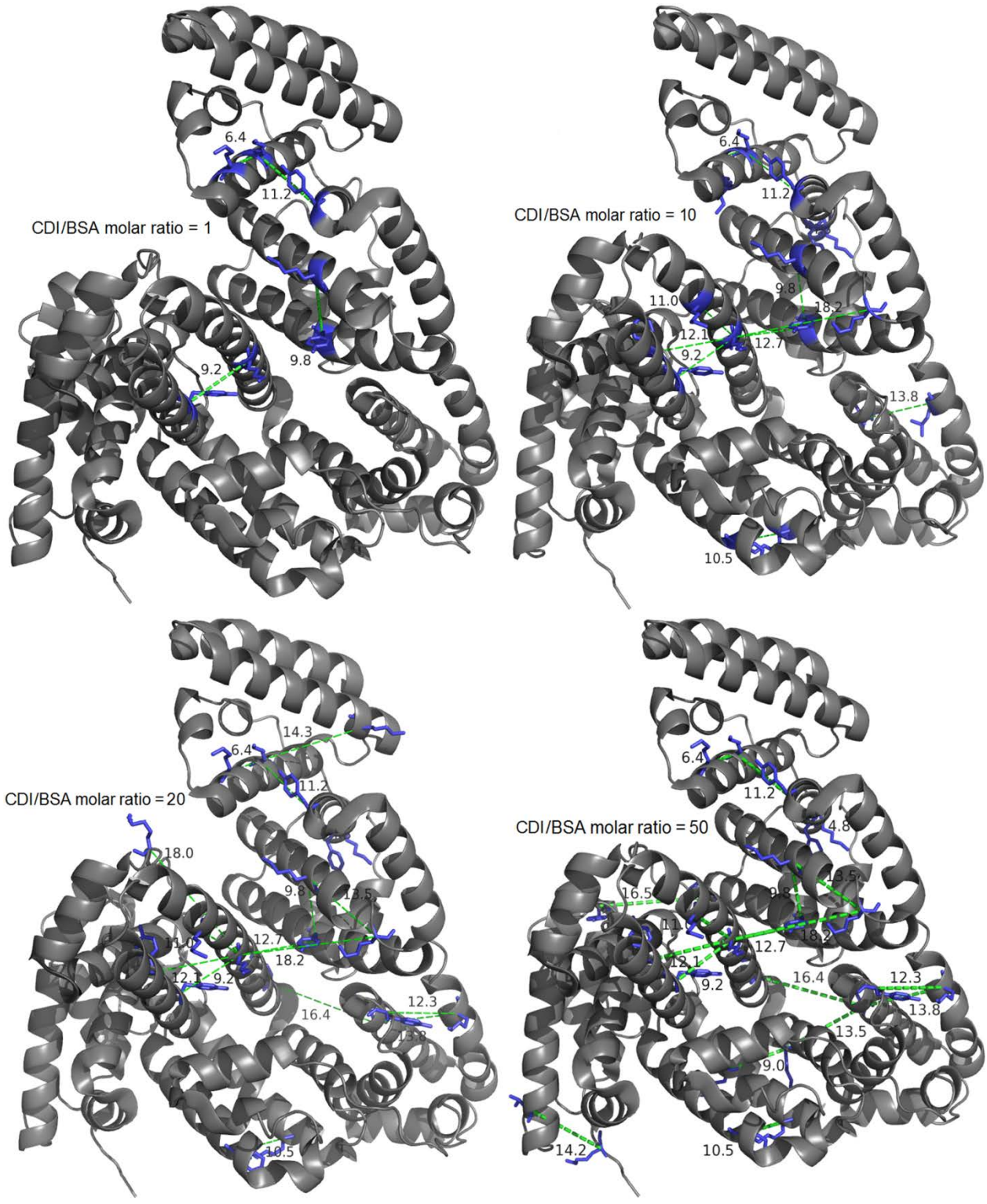

Figure S4: Cross-links created with different molar excess of CDI and mapped into the BSA structure (pdb 4F5S).

Table S2: Unique cross-links identified within BSA using DSBU. Numbering is performed according to the amino acid sequence of BSA as presented in Figure S1. Cross-links identified for consecutive amino acid sequences are listed separately as they are isobaric to 'dead-end' cross-links involving the same amino acids[56].

Replicate 1		Replicate 2		Replicate 3	
Cross-linked amino acid 1	**Cross-linked amino acid 2**	**Cross-linked amino acid 1**	**Cross-linked amino acid 2**	**Cross-linked amino acid 1**	**Cross-linked amino acid 2**
28	82	28	82	28	82
28	263	28	263	155	225
155	225	155	225	204	455
204	455	204	455	211	225
211	225	211	225	211	245
211	245	211	245	211	304
211	304	211	304	211	455
211	455	211	455	211	463
211	463	211	463	225	235
225	245	225	245	225	245
225	437	225	437	225	374
225	455	225	489	225	437
225	489	228	498	225	455
225	498	228	489	225	498
228	455	228	455	228	374
228	489	235	263	228	489
228	495	235	266	228	498
228	498	235	374	235	263
235	266	245	299	235	266
245	455	245	463	235	374
245	463	245	503	245	263
245	466	374	498	245	455
245	503	437	495	245	463
374	498	437	498	245	503
437	495	437	501	374	498
437	498	437	561	437	495
437	501	455	489	437	498
437	561	455	495	437	501
455	489	455	503	437	561
455	495	489	501	455	489
455	498			455	495
455	503			455	503
489	501			489	501
		Consecutive sequences			
28	36	28	36	28	36
29	36	204	211	204	211
204	211	211	245	204	455
235	245	211	455	235	245
235	263	228	503	235	263
297	304	235	245	297	304
455	463	235	263	455	463
		245	463	455	466
		297	304	455	503
		455	463		
		455	466		

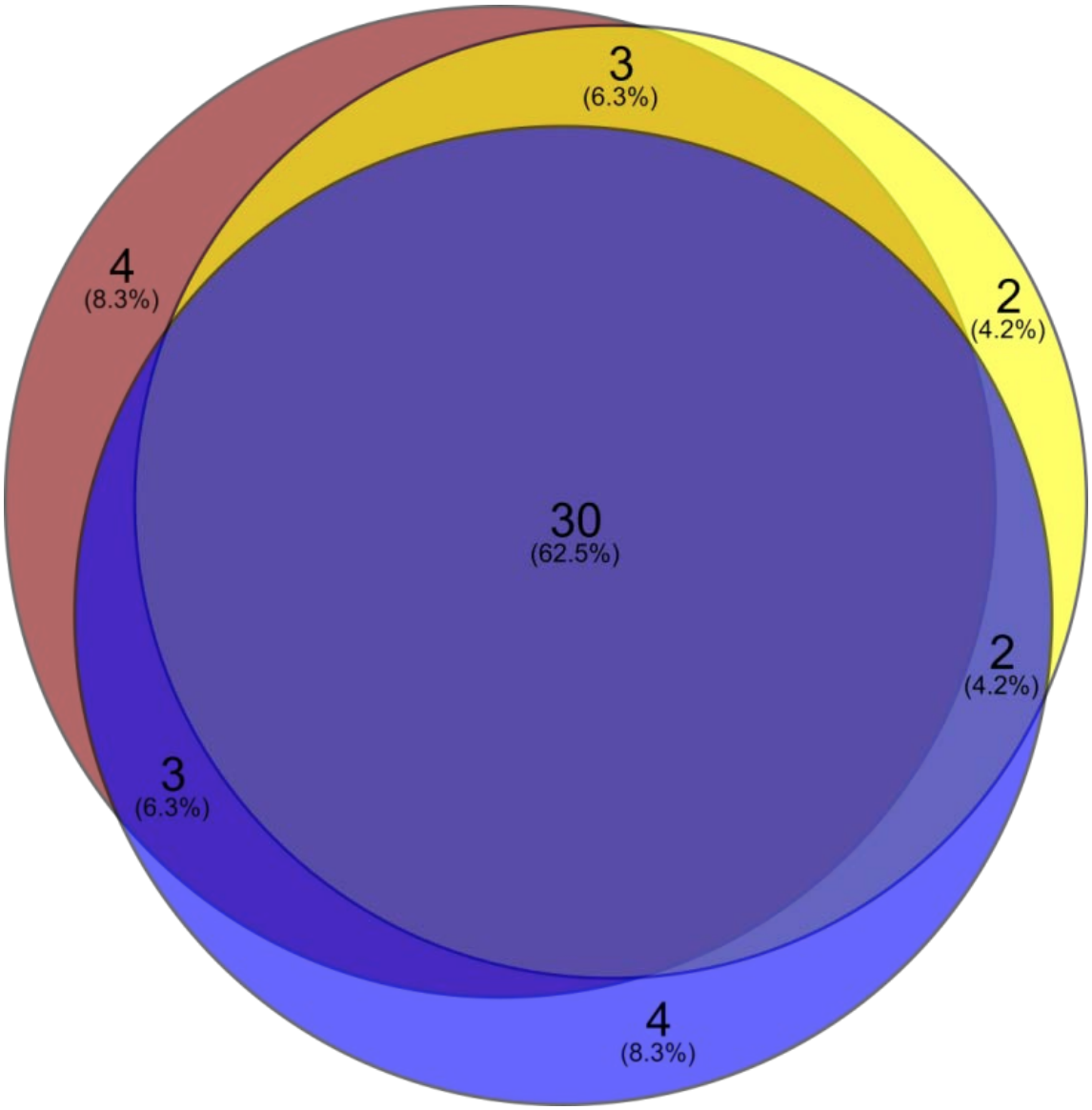

Figure S5: Reproducibility of BSA cross-linking experiments using DSBU. Comparison of unique cross-links as presented in Table S2.

Spectra assigned by MeroX

Exemplary spectra from BSA cross-linking using DSBU (replicate 1), assigned by the MeroX software are reported in Figure 6A-J. A-E. *Spectra are well annotated.* The cross-linking sites as well as the amino acid sequences of cross-linked peptides can be unambiguously assigned. Complete amino acid backbone fragmentation of both connected peptides as well as linker fragments are observed. F-J) *Spectra are inadequately annotated*. Cross-linked peptides cannot be unambiguously identified and the position of the cross-linked amino acid cannot be exactly localized. A brief description is given for each inadequately annotated spectrum. B indicates carbamidomethylated cysteine. Bu and BuUr specify DSBU fragments (see Figure 2).

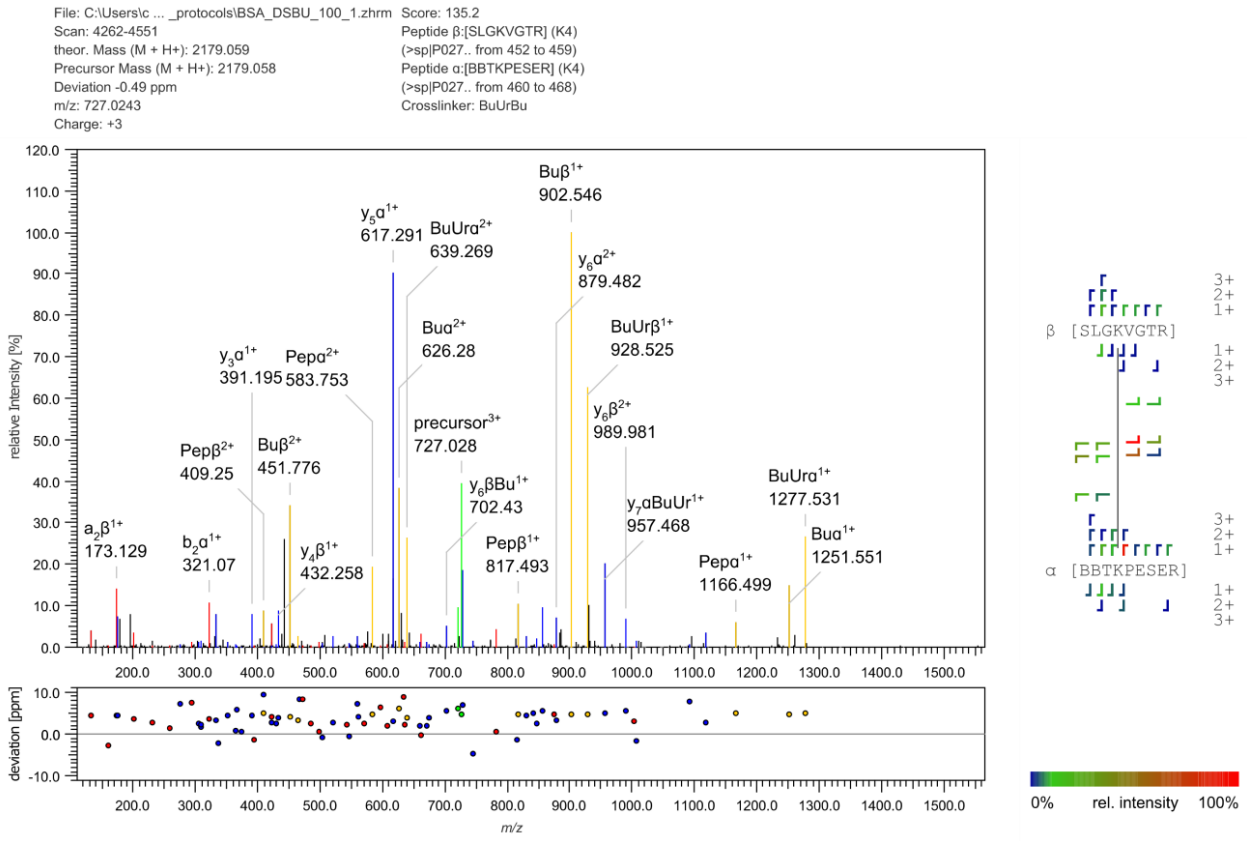

Figure S6A: The cross-linked amino acids are unambiguously assigned.

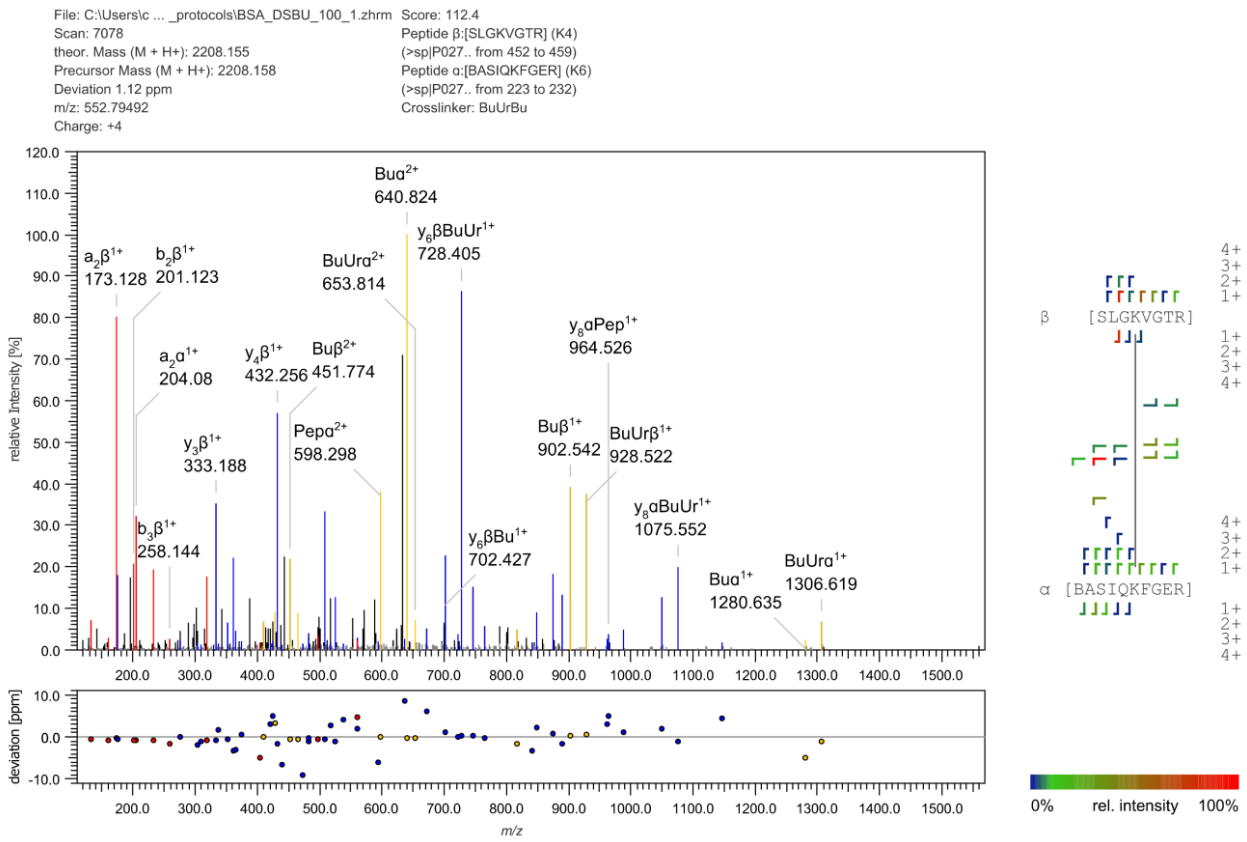

Figure S6B: The cross-linked amino acids are unambiguously assigned.

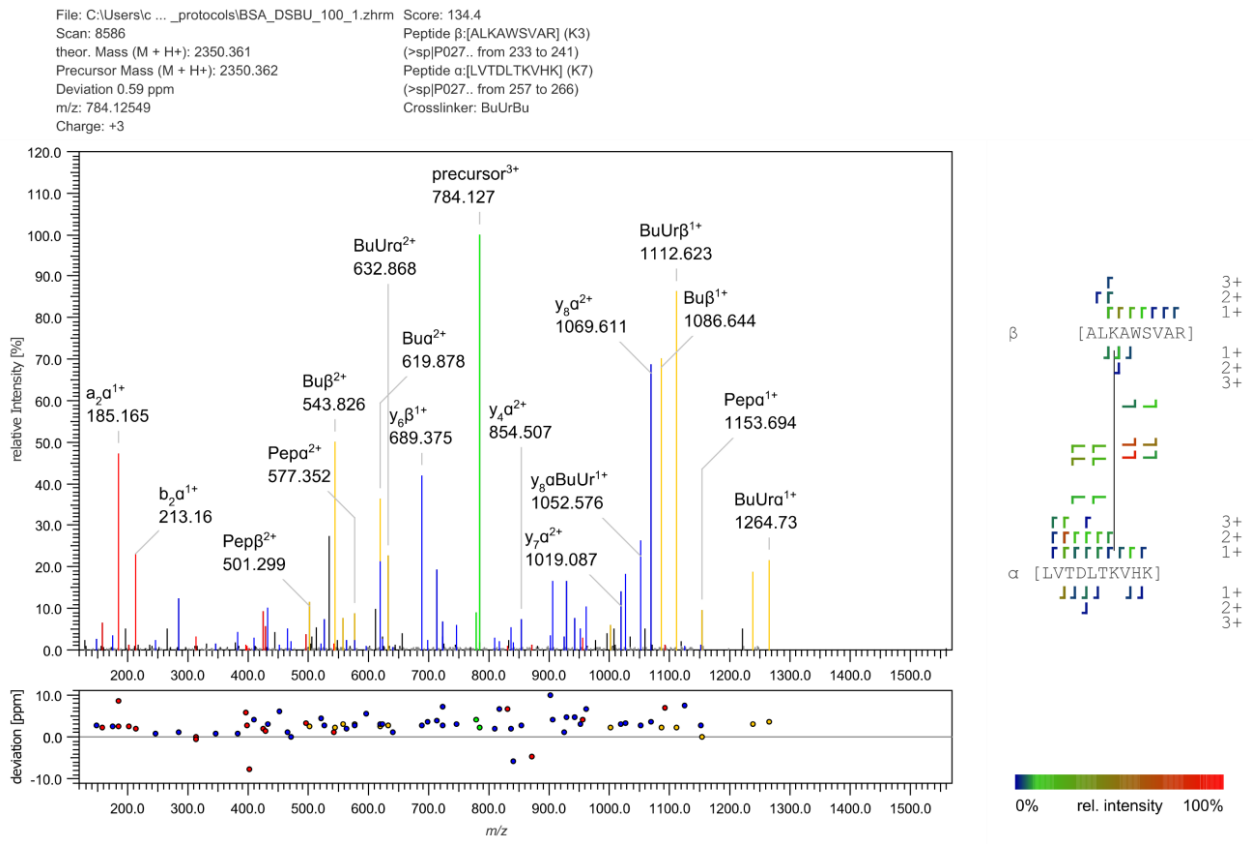

Figure S6C: The cross-linked amino acids are unambiguously assigned.

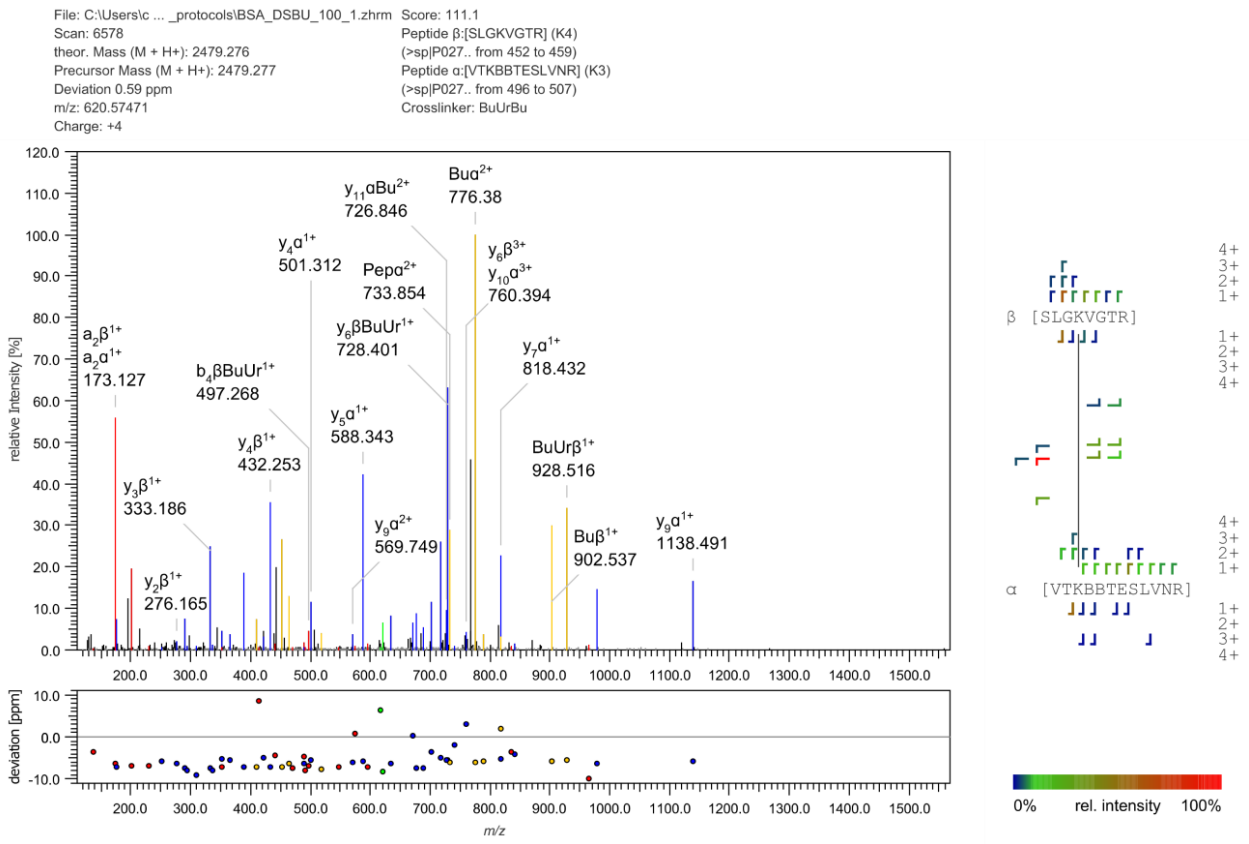

Figure S6D: The cross-linked amino acids are unambiguously assigned.

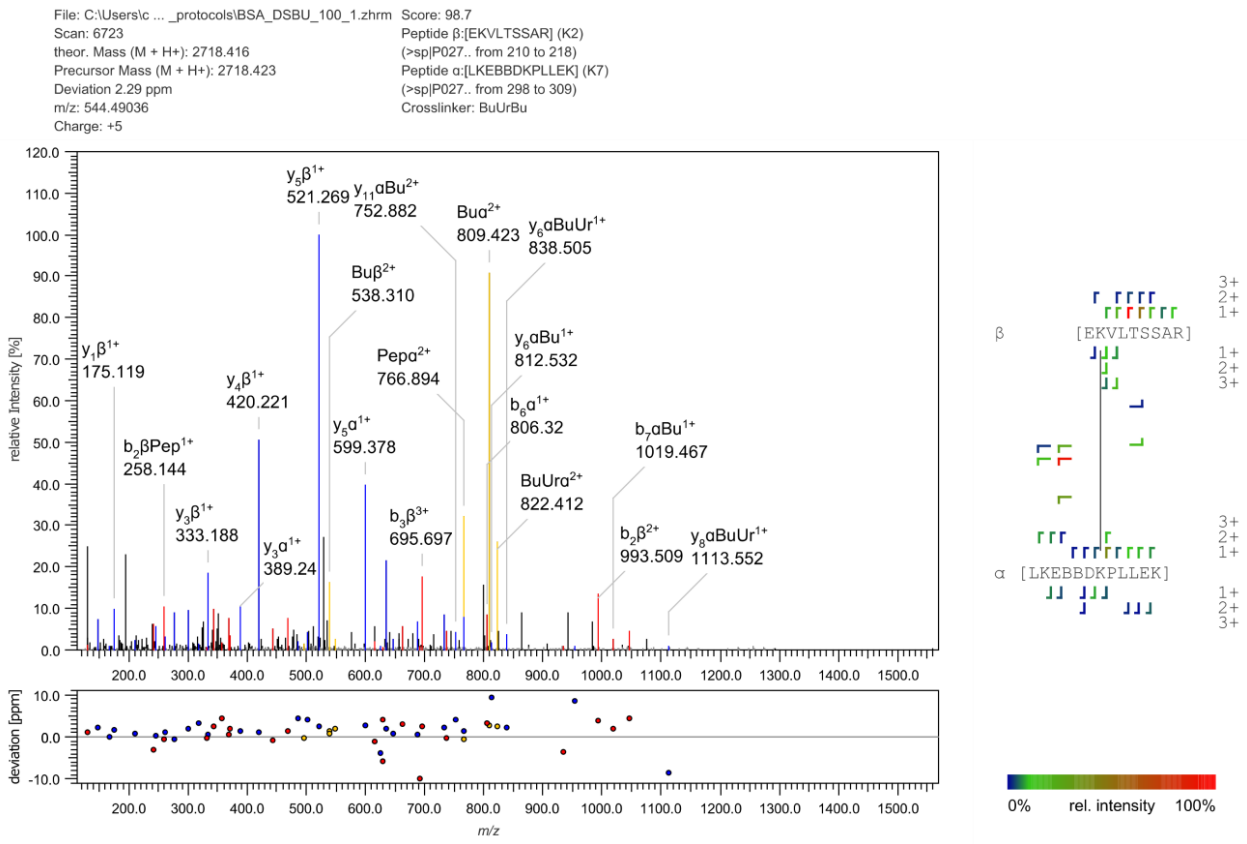

Figure S6E: The cross-linked amino acids are unambiguously assigned.

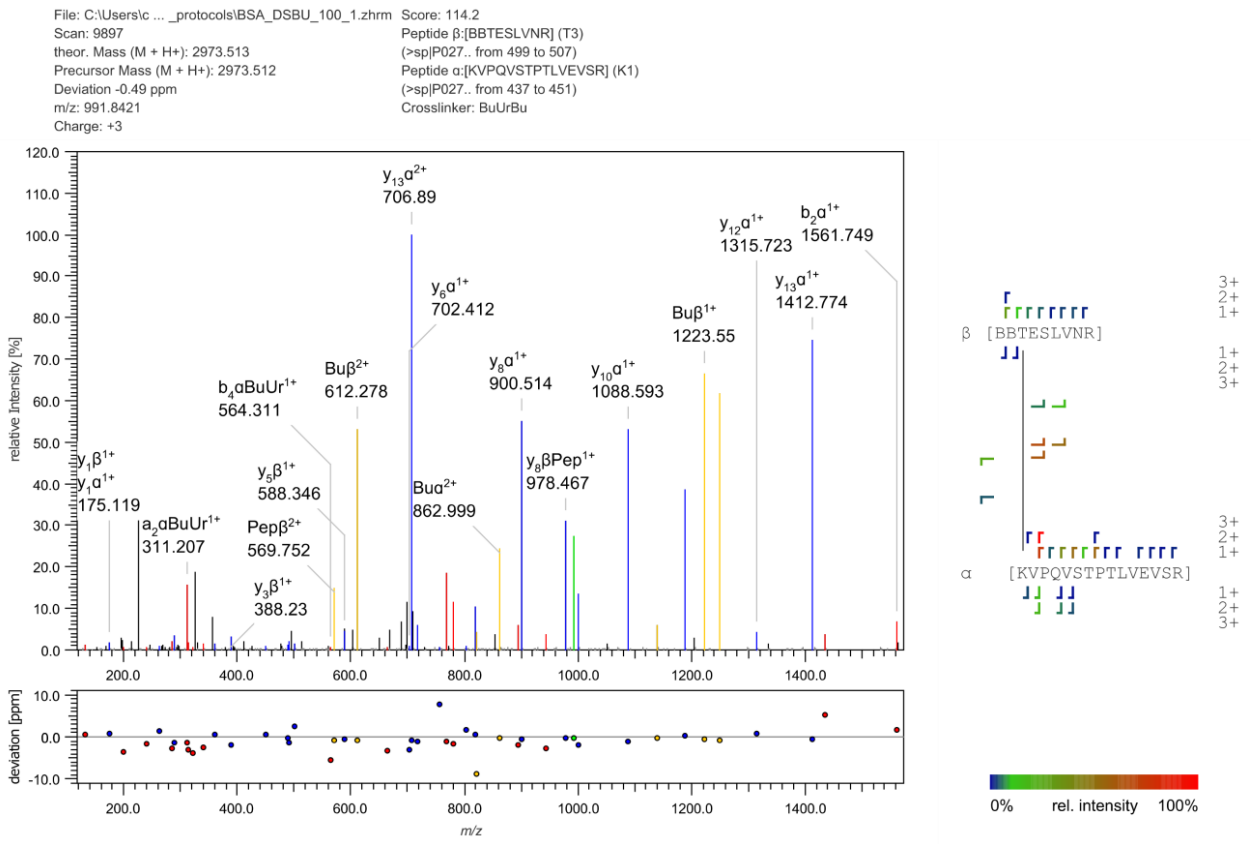

Figure S6F: The cross-linked amino acid in the β-peptide is not unambiguously assigned as relevant b-type ions pointing to the exact cross-linking position are missing.

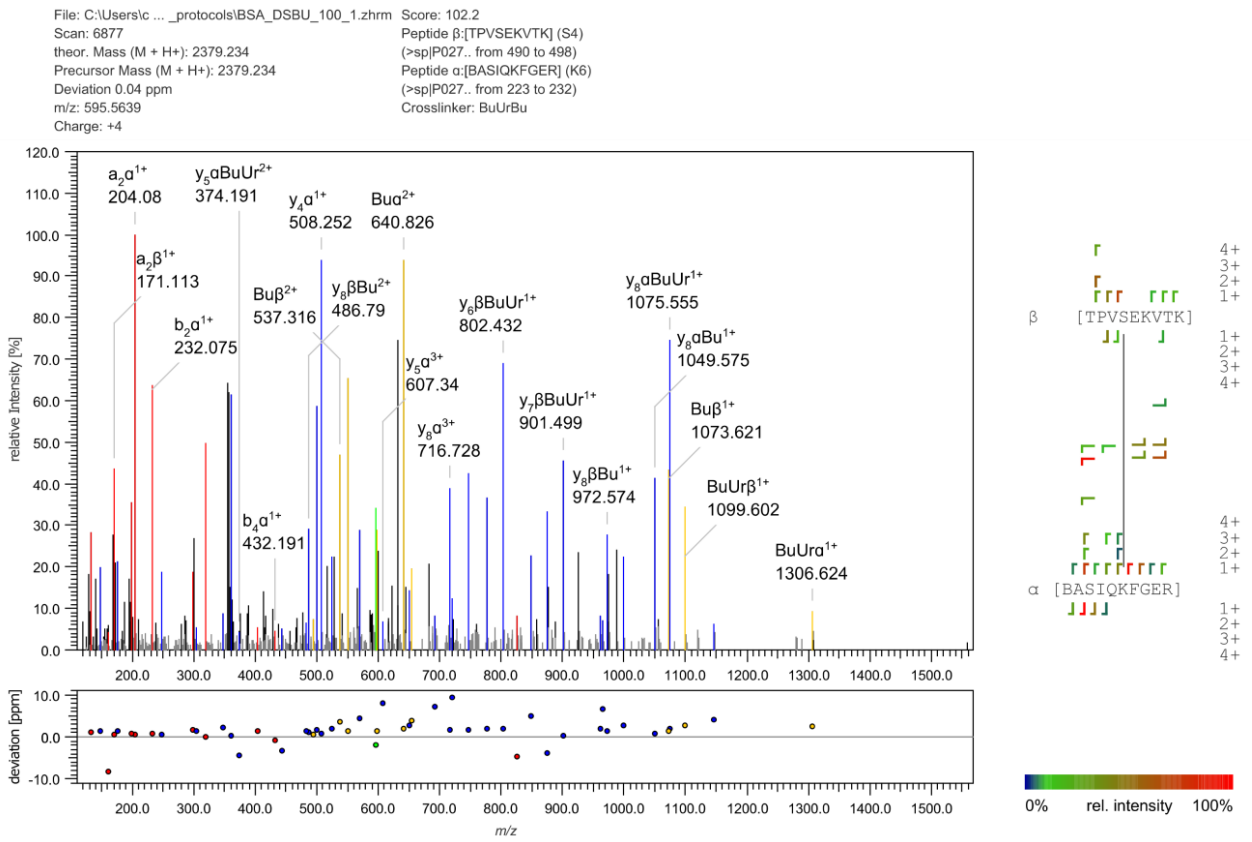

Figure S6G: The cross-linked amino acid in the β-peptide is not unambiguously assigned as relevant b- and y-type ions pointing to the exact cross-linking position are missing.

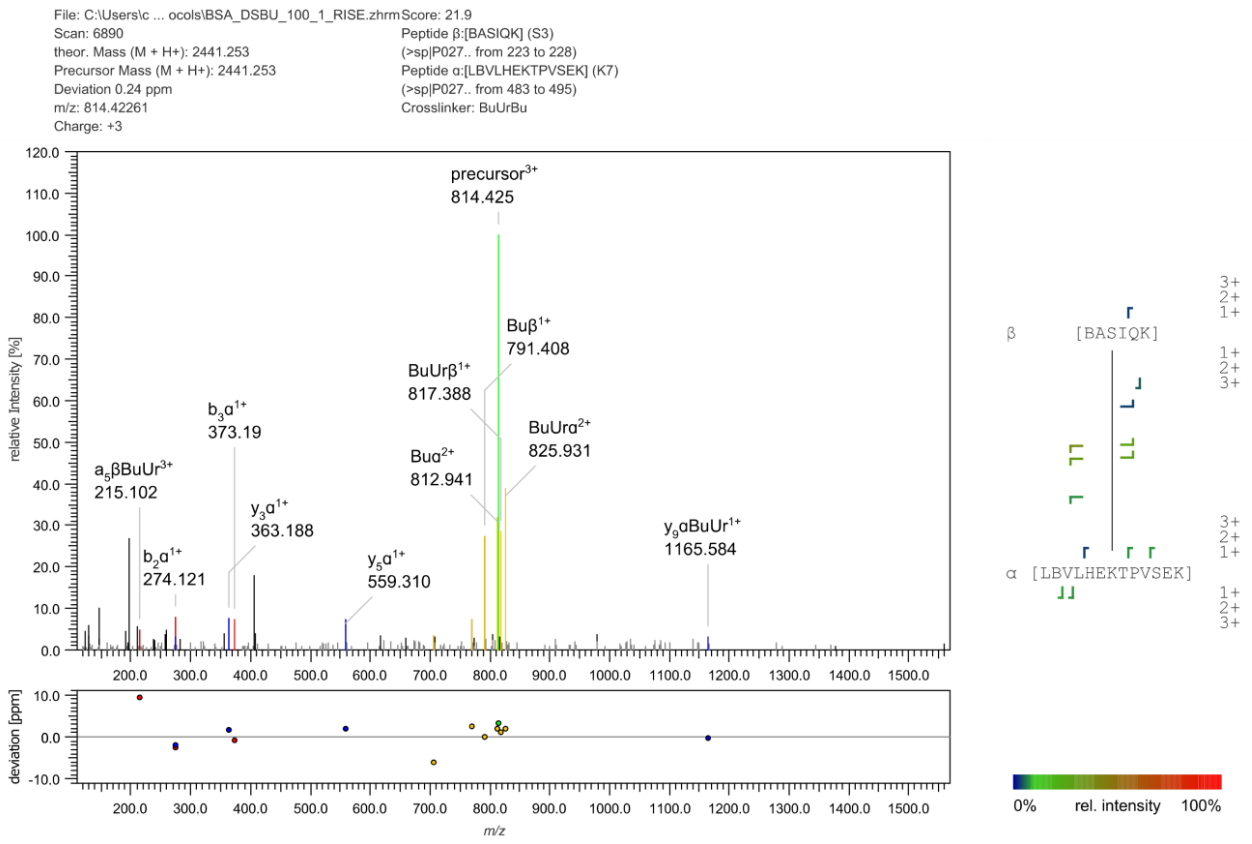

Figure S6H: The number of fragments identified for both peptides is not sufficient for an unambiguous identification of the amino acid sequences and the exact cross-linking positions. Only fragments of the DSBU are clearly visible.

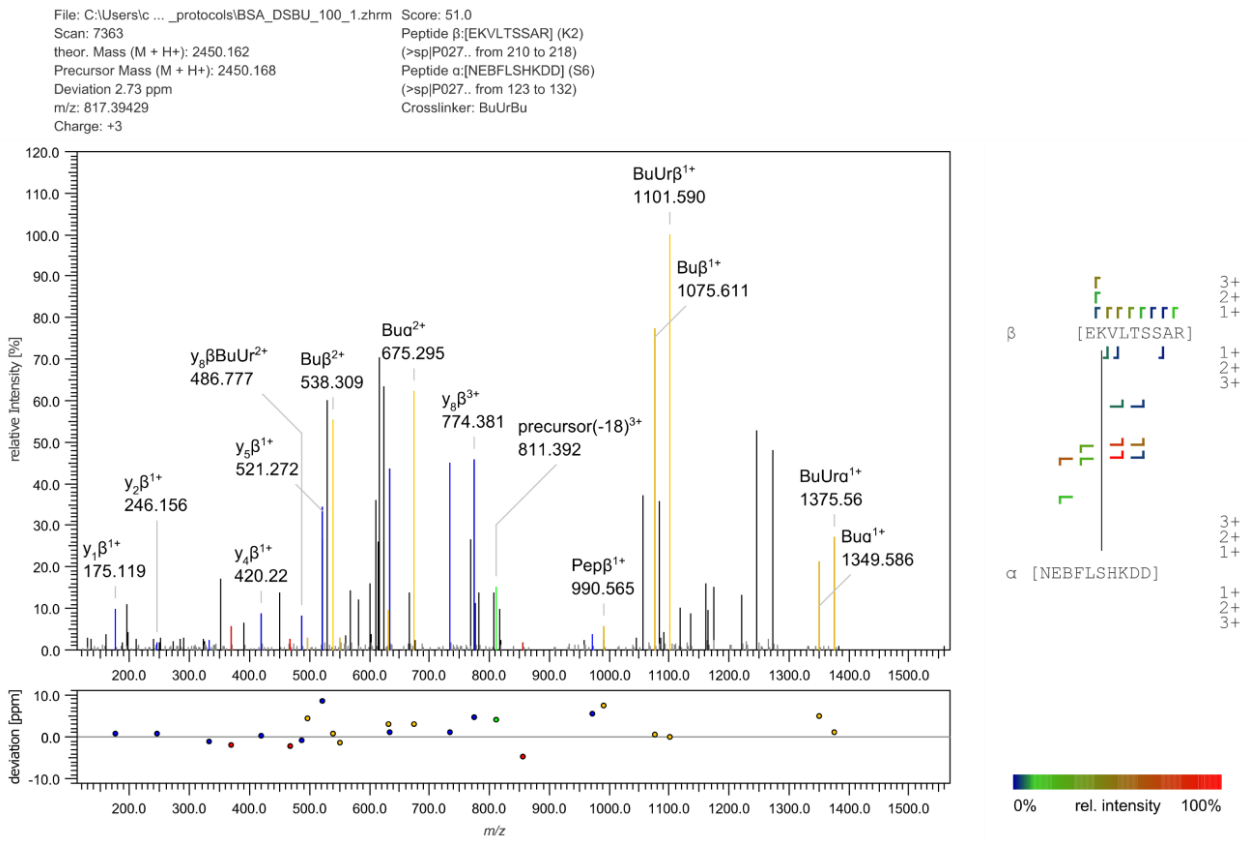

Figure S6I: No fragments are present for the α-peptide in the spectrum. Therefore, neither the amino acid sequence of the α-peptide, nor the exact cross-linking position can be unambiguously determined.

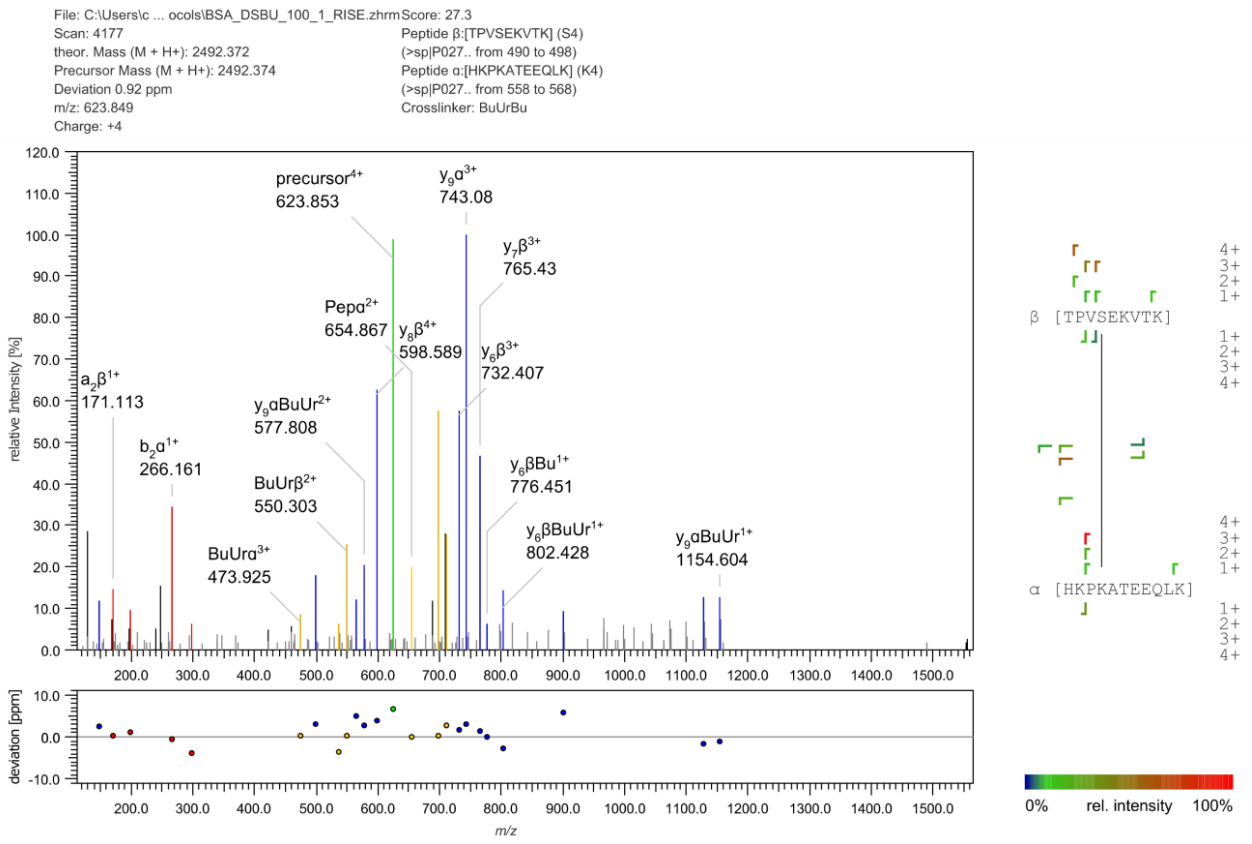

Figure S6J: The number of fragments identified for both peptides is not sufficient for an unambiguous identification of the amino acid sequences and the exact cross-linking positions.

Cross-linking of *E. coli* Ribosome

Experimental procedure

0.5 µM *E.coli* ribosome (1.25 mg/mL in 20 mM HEPES, pH 7.6) was cross-linked with 0.5 mM, 1 mM, 2.5 mM and 5 mM DSBU (corresponding to DSBU:lysine ratios of ~ 1, 2, 5 and 10) at room temperature for 60 min. The cross-linking reactions were quenched by adding TRIS buffer to a final concentration of 50 mM. *In-solution* digestion was conducted with trypsin using an enzyme-protein ratio of 1:50 at 37 °C overnight. After enrichment of cross-linking products using OASIS MCX cartridges, the resulting peptide mixtures were applied to LC/MS/MS analysis (Ultimate 3000 RSLC nano-HPLC system coupled to an Orbitrap Q-Exactive Plus mass spectrometer, both Thermo Fisher Scientific) applying stepped HCD (normalized collision energies 27 %, 30 %, and 33 %).

Cross-linked products were identified using MeroX with FDRs set to 5%, no manual validation of cross-linked products was performed. Mgf files were searched against a fasta file containing the amino acid sequences of *E.coli* ribosomal proteins (UniProt), MeroX settings were as follows: Protease: trypsin, max. 1 missed cleavage C-terminal of K and/or R, carbamidomethylation of C was considered as fixed modification, oxidation of M, and DSBU “dead-end” (type 0) cross-links at K were considered as variable modifications. Mass measurement accuracy was set to 3 ppm (MS) and 10 ppm for (MS/MS). RISE mode was enabled in MeroX and one missing DSBU fragment was allowed.

Table S3: 766 unique cross-links were identified with MeroX (5% FDR) within the *E. coli* ribosome. 0.5 µM *E.coli* ribosome (1.25 mg/mL in 20 mM HEPES, pH 7.6) was cross-linked with 0.5 mM, 1 mM, 2.5 mM and 5 mM DSBU (samples 1, 2, 5, 10) at room temperature for 60 min. Numbering was performed according to the amino acid sequences of *E. coli* ribosomal proteins of (UniProt), 0 corresponds to protein *N*-terminus. Cross-links identified for consecutive amino acid sequences were ignored.

Unique cross-linking site				Identified in sample			
Protein 1	**Amino acid 1**	**Protein 2**	**Amino acid 2**	**1**	**2**	**5**	**10**
RL1	54	RL1	167	x	x	x	x
RL1	54	RL1	163/167	x	x	x	x
RL1	167	RL1	210/211	x	x	x	
RL1	6	RL1	37			x	x
RL1	54	RL1	69			x	x
RL1	173	RL1	210/211	x		x	
RL1	6	RL1	14	x	x		
RL1	154	RL1	167	x	x		
RL1	141/144/146/154	RL1	198	x	x		
RL1	54/55	RL1	167	x	x		
RL1	54	RL1	210/211	x	x		
RL1	197/198	RL1	200	x	x		
RL1	54	RL1	163				x
RL1	54	RL1	63			x	
RL1	6	RL1	35/37			x	
RL1	154	RL1	198		x		
RL1	6	RL1	41		x		
RL1	54/55	RL1	163/167		x		
RL1	163/167	RL1	210/211		x		
RL1	154	RL1	163/167	x			
RL1	54	RL1	141	x			
RL1	54	RL1	163/167/173	x			
RL1	105	RL1	106	x			
RL1	141	RL1	198	x			
RL10	37	RL11	66	x	x	x	x
RL10	43	RL4	74/75	x	x	x	x
RL10	37	RL11	72	x	x	x	x
RL10	37/39	RL11	72	x	x		x
RL10	43	RL4	57/58			x	x
RL10	43	RL11	72		x		x
RL10	43	RL4	58		x		x
RL10	39	RL10	105		x	x	
RL10	37/39	RL10	105	x		x	

RL10	37/39/43	RL10	109	x	x		
RL10	37	RL10	43	x	x		
RL10	43	RL4	70/72/74				x
RL10	37	RL4	101			x	
RL10	58	RL16	133			x	
RL10	39	RL11	72			x	
RL10	43	RL4	55/57/58			x	
RL10	43	RL10	101		x		
RL10	43	RL11	66		x		
RL10	39/43	RL10	105		x		
RL10	97/101	RL10	109	x			
RL10	43	RL10	105	x			
RL10	43	RL7	71	x			
RL10	37/39	RL10	109	x			
RL10	37/39/43	RL10	105	x			
RL10	37/39	RL7	71	x			
RL10	97	RL10	109	x			
RL11	81/82	RL11	87/88/90/92		x		
RL11	81/82	RL11	87/88/90/92/95	x			
RL11	82	RL11	100	x			
RL13	23/24	RL13	61	x		x	x
RL13	23/24	RL13	61/65/68			x	x
RL13	23	RL13	68				x
RL13	23/24	RL13	65/68			x	
RL13	23	RL13	61			x	
RL13	12	RL13	41		x		
RL13	21/23/24	RL13	61/65/68		x		
RL13	23	RL13	65	x			
RL14	59	RL14	111	x	x	x	x
RL14	75	RL16	133		x	x	x
RL14	44	RL14	59	x		x	x
RL14	40/42/44	RL14	59		x	x	
RL14	40	RL14	59	x			
RL15	39/40	RL15	63		x	x	x
RL15	17	RL22	98			x	x
RL15	84	RL4	99			x	x
RL15	80	RL4	99	x	x		
RL15	39	RL15	63				x
RL15	84	RL15	141				x
RL15	17	RL4	55/57/58		x		
RL15	14	RS15	71		x		
RL16	8	RL16	11/14		x	x	x

RL16	62	RL16	133	x	x		x
RL16	8	RL16	58	x	x	x	
RL16	58	RL16	62		x		x
RL16	11/14	RL16	84/86		x	x	
RL16	34	RL16	132/133		x	x	
RL16	5	RL16	8		x	x	
RL16	58	RL16	123	x			x
RL16	118	RL16	127	x			x
RL16	58	RL16	118	x		x	
RL16	14	RL16	84/86	x	x		
RL16	8	RL16	84/86				x
RL16	62	RS4	185				x
RL16	34	RL16	127				x
RL16	14	RL16	84				x
RL16	123	RL16	127/128/129			x	
RL16	34	RL16	133			x	
RL16	62	RL16	133/134			x	
RL16	62	RL16	128			x	
RL16	11	RL16	84			x	
RL16	118/123	RL16	127/128/129/132/133		x		
RL16	11/14	RL16	84		x		
RL16	118/123	RL16	127/128/129		x		
RL16	58	RL16	118/123		x		
RL17	35/36/37	RL32	53	x	x	x	x
RL17	35	RL32	53	x	x	x	x
RL17	35	RL32	57	x	x	x	x
RL17	78	RL17	121		x	x	
RL17	78	RL17	119/121	x			x
RL17	35/36/37	RL32	57				x
RL17	35/36/37/40	RL32	57			x	
RL17	35/36	RL32	53		x		
RL17	40	RL32	53	x			
RL18	17	RL27	62	x	x	x	x
RL18	17	RL27	62/66	x	x	x	x
RL18	68	RL18	76	x	x	x	x
RL18	76	RL18	88	x	x	x	x
RL18	63	RL18	76	x	x	x	
RL18	17	RL27	75			x	x
RL18	63/64/65/68	RL18	76	x		x	
RL18	4	RL27	78				x
RL18	24	RL27	62/66				x

RL18	4/5	RL27	75			x	
RL18	0	RL27	75			x	
RL18	0/3	RL27	75		x		
RL18	4	RL27	75		x		
RL18	63/64/65/68	RL31	69/70		x		
RL18	88	RS1	278/279/281	x			
RL18	17	RL27	66	x			
RL18	88	RS1	279/281	x			
RL18	63	RL31	69/70	x			
RL18	0/3	RL5	161/162	x			
RL18	63/64/65	RL18	76	x			
RL19	87	RL19	111	x	x	x	x
RL19	63	RL19	87	x	x	x	x
RL19	87	RL19	106	x	x	x	x
RL19	83	RL19	111		x	x	x
RL19	106	RL19	111	x	x		x
RL19	63	RL19	85/87				x
RL19	65	RL19	87				x
RL19	96	RL19	111			x	
RL19	85	RL19	106	x			
RL2	183	RL2	265	x	x	x	x
RL2	97	RL2	125	x	x	x	x
RL2	108/111	RL2	157			x	x
RL2	108/111	RL2	125	x			x
RL2	96/97	RL2	125	x			x
RL2	108	RL2	125	x		x	
RL2	97	RL2	125/129				x
RL2	261	RL2	265				x
RL2	7/9/10	RL2	97			x	
RL2	68	RL9	89/93/96		x		
RL2	108/111	RL2	125/129		x		
RL2	18	RL31	62		x		
RL2	111	RS1	200		x		
RL2	108	RL2	157		x		
RL2	9/10	RL2	97		x		
RL2	261	RS1	511	x			
RL2	111	RL2	157	x			
RL20	78/84	RL20	85	x	x	x	x
RL20	85	RL20	114	x	x	x	x
RL20	22	RL21	73			x	x
RL20	16	RL31	62		x		x
RL20	16	RS14	50	x		x	

RL20	16	RL21	73/76				x
RL20	77/78/84	RL20	85				x
RL20	16	RL21	73				x
RL20	103	RL20	114			x	
RL21	0	RL13	12		x	x	x
RL21	0	RL21	76		x	x	x
RL21	7/10	RL21	60		x	x	
RL21	0	RL21	15	x		x	
RL21	0	RL13	2	x	x		
RL21	10	RL20	22				x
RL21	2	RL13	12			x	
RL21	0	RL13	7/10/12			x	
RL21	0	RL13	7		x		
RL21	0	RL13	10/12		x		
RL21	7	RL21	60		x		
RL21	0	RL13	0/2	x			
RL21	10	RL21	76	x			
RL21	0	RL13	0	x			
RL21	10	RL21	60	x			
RL22	0	RL22	81/83		x	x	x
RL22	48/49	RL32	57		x	x	x
RL22	0	RL22	73	x	x		x
RL22	6	RL22	73	x	x	x	
RL22	0	RL22	81		x	x	
RL22	6	RL22	73/81/83		x	x	
RL22	6	RL22	72/73	x		x	
RL22	28	RL32	37	x	x		
RL22	0	RL22	72			x	
RL22	3	RL22	83			x	
RL22	42	RL32	57			x	
RL22	0	RL22	72/73		x		
RL22	6	RL22	72		x		
RL22	16	RL22	98	x			
RL22	6	RL22	81	x			
RL22	28	RL22	70	x			
RL22	28	RL22	70/72/73	x			
RL22	6	RL22	83	x			
RL22	6	RL22	81/83	x			
RL23	0	RL23	9	x	x	x	x
RL23	9	RL29	54	x	x	x	x
RL23	9	RL23	36		x	x	x
RL23	9	RL23	39/40	x		x	x

RL23	0	RL23	49	x	x		
RL23	9	RS12	15	x	x		
RL23	0	RL29	54				x
RL23	9	RL23	26/27/29/33			x	
RL23	0	RL36	18			x	
RL23	27/29/33	RL23	82			x	
RL23	9	RL29	60			x	
RL23	26/27/29/33	RL23	82		x		
RL23	9	RL23	36/39/40	x			
RL24	21	RL24	43/44	x	x	x	x
RL24	26	RL24	44	x	x	x	
RL24	91/92	RL24	104			x	x
RL24	43/44	RL24	47	x		x	
RL24	26	RL24	61	x	x		
RL24	26/30/31	RL24	104				x
RL24	21	RS5	22/23			x	
RL24	31	RL24	61		x		
RL24	24	RL24	44		x		
RL24 and RL23 @65-68 and RL14 @50-53	51	RL14	114	x	x	x	x
RL25	0	RL25	73	x	x	x	x
RL25	83	RL16	127	x	x	x	x
RL25	83	RL16	127/128/129/132/133		x	x	x
RL25	14	RL25	25	x	x	x	
RL25	83	RL16	118		x	x	
RL25	83/85	RL16	118	x			x
RL25	0	RL25	53	x	x		
RL25	25	RL25	53	x	x		
RL25	83	RL16	127/128/129	x	x		
RL25	0	RL25	71	x	x		
RL25	34	RL25	73	x	x		
RL25	3	RL25	46	x	x		
RL25	25	RS15	71				x
RL25	83	RL10	147		x		
RL25	0/3	RL25	53	x			
RL25	34	RL25	71	x			
RL25	0	RL7	71	x			
RL27	66	RL27	72	x	x	x	x
RL27	62	RL27	72	x	x	x	x
RL27	62/66	RL27	72	x	x	x	x

RL27	62	RL27	72/75		x	x	x
RL27	5	RL5	78	x	x		
RL27	66	RL27	75				x
RL27	75	RS4	156			x	
RL27	24	RL2	111			x	
RL27	58/62	RL27	75	x			
RL27	66	RL27	72/75	x			
RL27	62/66	RL27	72/75	x			
RL28	62	RL28	77/78	x	x	x	x
RL28	44	RL9	57	x	x	x	x
RL28	44	RL9	112/113	x		x	x
RL28	44	RL28	77/78			x	x
RL28	54	RL28	62			x	x
RL28	44	RL9	71	x		x	
RL28	44	RL9	112		x		
RL29	9	RL10	58	x	x		x
RL29	16	RL29	60		x	x	
RL29	9	RL4	65				x
RL29	4	RL29	16			x	
RL29	4	RS8	105		x		
RL29	16	RL15	141		x		
RL29	2	RL29	16	x			
RL29	9	RL15	141	x			
RL3	7/8	RS18 and RL3 @56-59	56	x	x	x	x
RL3	55/56	RL3	70		x	x	x
RL3	55/56	RL3	62		x	x	x
RL3	7/8	RL3	55/56		x	x	x
RL3	0	RL3	105/106			x	x
RL3	105/106	RL3	190				x
RL3	105/106	RL3	208				x
RL3	0	RL3	55/56				x
RL3	0	RS18 and RL3 @56-59	56				x
RL3	0	RL3	208		x		
RL3	7/8	RL3	38	x			
RL30	56	RS14	83			x	
RL30	12	RS20	19			x	
RL30	6	RL30	56			x	
RL30	56	RL20	84/85		x		
RL30	10	RL30	56		x		
RL31	62	RS13	110	x	x	x	x

RL31	62	RL5	120/121	x	x	x	
RL31	69/70	RL5	161/162		x		x
RL31	69/70	RL5	120		x	x	
RL31	69/70	RL5	78	x		x	
RL31	23	RL31	69/70	x	x		
RL31	62	RS13	62		x		
RL31	62	RL5	121	x			
RL31	62	RL5	78	x			
RL31	8	RL5	64/68/69	x			
RL31	23	RL5	121	x			
RL31	23	RL31	45/47	x			
RL31	69/70	RL5	145	x			
RL31	70	RL5	121	x			
RL32	32/33/34/37	RL17	119/121	x	x	x	x
RL32	32/33/34/37	RL32	57	x	x	x	x
RL32	32	RL17	119/121	x	x	x	x
RL32	37	RL22	48/49	x	x	x	x
RL32	32/33/34/37	RL32	53	x	x	x	
RL32	32	RL32	57		x		x
RL32	32	RL17	119		x	x	
RL32	32/33/34/37	RL17	121	x			x
RL32	18	RL32	57	x	x		
RL32	32/33	RL17	121				x
RL32	11	RS19	17/18			x	
RL32	32/33/34	RL17	121			x	
RL32	32/33/34	RL32	53		x		
RL32	34/37	RL32	53		x		
RL32	57	RL17	78		x		
RL32	32/33/34	RL32	57	x			
RL32	32/33/34	RL17	42	x			
RL32	33/34	RL17	121	x			
RL32	37	RL17	119	x			
RL32	33	RL17	121	x			
RL33	10	RL33	37	x	x	x	x
RL33	10	RL22	90	x	x		
RL33	10	RL33	53	x	x		
RL33	10	RL33	53/55	x	x		
RL33	10	RS4	23				x
RL33	10	RL33	33/37			x	
RL33	10	RS4 and RS1 @87-90	90		x		
RL33	10	RL21	60	x			

RL33	10	RL1	198	x			
RL33	10	RS4 and RS1 @87-90	88	x			
RL33	10	RL33	33	x			
RL34	11	RL34	45/46	x		x	x
RL35	23	RL35	51/52			x	x
RL35	16	RL35	35/36			x	x
RL35	36	RL2	157				x
RL35	16	RS9	129			x	
RL35	5	RL29	16			x	
RL35	12	RS9	129			x	
RL36	9	RL36	18	x	x	x	x
RL36	9	RL16	118	x	x	x	x
RL36	9	RL36	15	x	x	x	x
RL36	9	RL16	118/123	x	x	x	
RL36	6/8	RS21	54	x		x	
RL36	9	RL36	15/18				x
RL36	9	RL16	123				x
RL36	6	RS12	108		x		
RL36_2	21	RL21	73		x	x	x
RL36_2 and RL36_2 @44-47	44	RL2	108	x			
RL4	0	RL4	137	x	x	x	x
RL4	99	RL4	106	x	x	x	x
RL4	130	RL4	137	x	x	x	x
RL4	65	RL4	74		x	x	x
RL4	58	RL4	74/75		x	x	x
RL4	98/99	RL4	106		x	x	x
RL4	57/58	RL4	74/75	x	x		x
RL4	123	RL4	194	x	x	x	
RL4	0	RL4	137/139	x	x	x	
RL4	6	RL4	137	x	x	x	
RL4	123	RL4	137	x	x	x	
RL4	47/48	RL4	74/75			x	x
RL4	57/58	RL4	74			x	x
RL4	130	RL4	137/139			x	x
RL4	58	RL4	65			x	x
RL4	6	RL4	189	x			x
RL4	6	RL4	185/189	x	x		
RL4	58	RL4	63/65	x	x		
RL4	130	RL4	139	x	x		
RL4	130/131/132	RL4	137	x	x		

RL4	99	RL4	107				x
RL4	101	RL4	106				x
RL4	123/125	RL4	137				x
RL4	58	RL4	70				x
RL4	47	RL4	55			x	
RL4	0	RL4	185/189			x	
RL4	55/57/58	RL4	63			x	
RL4	132	RL4	166			x	
RL4	166	RL4	185			x	
RL4	99	RL4	107/110			x	
RL4	55/57/58	RL34 and RL22 @89-92	90		x		
RL4	6	RL4	137/139		x		
RL4	0/6	RL4	185/189		x		
RL4	55/57/58	RL4	74		x		
RL4	0/6	RL4	137		x		
RL4	57/58	RL4	63/65		x		
RL4	10/13/15/17/18	RL4	137		x		
RL4	58	RL4	74		x		
RL4	106	RL4	194	x			
RL4	123	RL4	139	x			
RL4	57/58	RL4	63	x			
RL4	130/131/132	RL4	137/139	x			
RL4	10	RL4	137	x			
RL5	47/48	RL5	78	x	x	x	x
RL5	78	RS13	102	x	x	x	x
RL5	78	RS13	110	x	x	x	x
RL5	47/48	RL5	69	x	x	x	
RL5	120	RL5	179			x	x
RL5	78	RS20 and RL19 @107-111	111	x		x	
RL5	15	RL5	120/121	x	x		
RL5	47/48	RL5	64/68/69	x	x		
RL5	15	RL31	69/70	x	x		
RL5	47/48	RL5	64	x	x		
RL5	73	RS7	76				x
RL5	78	RL5	83			x	
RL5	47/48	RL5	68		x		
RL5	78	RS13	103/104		x		
RL5	47/48	RL5	68/69		x		
RL5	15	RL31	62	x			

RL5	15	RL5	121	x			
RL5	78	RS13	117/118	x			
RL5	78	RS19	87/88	x			
RL6	6	RL6	44	x	x	x	x
RL6	86	RL6	138	x	x	x	x
RL6	85/86	RL6	138	x	x	x	
RL6	6	RL6	44/49/51	x		x	
RL6	29	RL7	71	x	x		
RL6	34	RL19	87			x	
RL6	86	RL7	90/96		x		
RL6	18/25/27	RL6	29	x			
RL6	86	RL7	109	x			
RL6	86	RL7	96	x			
RL6	18/25/27	RL6	44	x			
RL6	29	RL6	94	x			
RL6	44/49/51	RL7	71	x			
RL7	71	RL6	86	x	x	x	
RL7	85	RL6	86	x	x	x	
RL7	71	RL6	160	x	x		
RL7	71	RL7	71	x	x		
RL7	71	RL7	85	x	x		
RL7	66	RL7	71	x			
RL7	71	RL11	82	x			
RL7	66	RL10	97	x			
RL7	108	RL7	109	x			
RL7	71	RL10	105	x			
RL9	8	RL9	57	x	x	x	x
RL9	35	RL9	57	x	x	x	x
RL9	42	RL28	62	x	x	x	x
RL9	42	RL9	57	x	x	x	x
RL9	42	RL28	44	x	x	x	x
RL9	57	RL9	113		x	x	x
RL9	8	RL9	112/113	x		x	x
RL9	0	RL9	25	x	x		x
RL9	8	RL9	35	x	x	x	
RL9	0	RL9	22	x	x	x	
RL9	57	RL9	71	x	x	x	
RL9	0	RL9	22/25		x		x
RL9	35	RL9	113		x	x	
RL9	35/40/41	RL9	57	x		x	
RL9	0	RL9	57	x	x		
RL9	8	RL9	71	x	x		

RL9	42	RL28	61/62	x	x		
RL9	89	RL2	183	x	x		
RL9	89	RL2	97	x	x		
RL9	112/113	RL2	265				x
RL9	22	RL9	112/113				x
RL9	57	RL9	112/113			x	
RL9	0	RL28	61/62			x	
RL9	0	RL9	35		x		
RL9	22	RL9	35/40/41		x		
RL9	22	RL9	35		x		
RL9	83	RL2	97		x		
RL9	89	RL9	113		x		
RL9	89	RL9	112/113		x		
RL9	35/41	RL9	113		x		
RL9	35	RL28	44		x		
RL9	89	RS1	278/279/281	x			
RL9	14	RL9	71	x			
RL9	8	RL9	35/40/41	x			
RL9	22	RL9	57	x			
RL9	35	RL9	112/113	x			
RL9	41	RL9	113	x			
RL9	89	RL2	125	x			
RL9	89	RL9	112	x			
RL9	40/41	RL9	57	x			
RL9	35/40/41	RL28	44	x			
RL9	41/42	RL28	62	x			
RL9	42	RL9	112	x			
RS1	12/14	RS2	45	x	x		
RS1	279/281	RS1	350	x	x		
RS1	117	RS1	162		x		
RS1	14	RS2	20		x		
RS1	279	RS1	350		x		
RS1	12/14	RS4	177	x			
RS1	229	RS1	272	x			
RS1	247	RS1	256	x			
RS1	229	RS1	260	x			
RS1	150	RS1	256/260	x			
RS1	430/434	RS1	450	x			
RS1	370	RS1	411	x			
RS1	155	RS1	247	x			
RS1	278	RS1	350	x			
RS1	150	RS1	196/200	x			

RS1	150	RS1	247	x			
RS1	247	RS1	260	x			
RS1	450	RS1	504	x			
RS10	0	RS3	62/63	x	x	x	x
RS10	30	RS10	83	x	x	x	x
RS10	83	RS9	100		x	x	x
RS10	0	RS10	83	x	x	x	
RS10	11	RS14	76			x	x
RS10	30	RS10	82/83			x	x
RS10	0	RS10	82/83	x			x
RS10	30	RS9	100	x			x
RS10	82/83	RS1	504/506	x	x		
RS10	82/83	RS9	100				x
RS10	11	RS10	30				x
RS10	11	RS3	62/63		x		
RS10	82/83	RS1	536	x			
RS10	83	RS1	464	x			
RS11	14	RS11	87	x	x	x	
RS11	14	RL9	89	x	x	x	
RS11	0/3	RL16	8			x	x
RS11	17	RS11	80		x	x	
RS11	87	RS7	154	x		x	
RS11	0	RL16	132/133/134				x
RS11	0/3	RS13	110			x	
RS11	0	RS13	110			x	
RS11	87	RS7	149/154			x	
RS11	0/3	RL27	5		x		
RS11	87	RS7	149		x		
RS11	80	RS7	149/154		x		
RS11	14	RL9	83	x			
RS11	35	RS11	80	x			
RS12	108	RS12	123	x	x	x	x
RS12	111	RS12	123	x	x	x	x
RS12	44	RS12	51	x	x	x	x
RS12	108	RS12	111	x	x		x
RS12	44	RS21	59	x	x		
RS12	111	RL19	111				x
RS12	51	RS12	108			x	
RS12	15	RS18	38	x			
RS13	110	RS9	129	x	x	x	x
RS13	78	RS13	110	x	x	x	x
RS13	78	RL5	120/121	x	x	x	x

RS13	110	RL5	120/121	x	x	x	x
RS13	78	RL5	120	x	x	x	x
RS13	110	RS20 and RL19 @107-111	111		x	x	x
RS13	13	RS7	114		x	x	x
RS13	78	RS13	103/104		x	x	x
RS13	27/28	RS13	30/31	x	x		x
RS13	104	RL5	120			x	x
RS13	103	RL5	120/121		x		x
RS13	78	RS13	103		x	x	
RS13	103/104	RS13	110	x			x
RS13	55	RS7	114	x		x	
RS13	44	RS7	114				x
RS13	78	RS13	102				x
RS13	13	RL5	145				x
RS13	13	RS13	55				x
RS13	27	RS13	31			x	
RS13	44	RS7	114/115			x	
RS13	27/28	RS13	31			x	
RS13	27	RS13	30/31		x		
RS13	114	RL5	120/121	x			
RS13	110	RS7	114/115	x			
RS13	103/104	RS13	114	x			
RS13	62	RS7	114	x			
RS13	44/46/49	RS7	114/115	x			
RS13	117/118	RL5	120/121	x			
RS14	23	RS14	50	x	x	x	x
RS14	50	RL31	70		x	x	x
RS14	47	RL31	69/70	x		x	x
RS14	23	RS14	47		x	x	
RS14	12	RS3	89	x	x		
RS14	47/50	RL31	69/70	x	x		
RS14	76	RS14	83				x
RS14	19	RS14	47			x	
RS15	71	RS17	81	x	x	x	x
RS15	71	RS15	73	x	x	x	x
RS15	71	RL13	106		x	x	x
RS16	46	RS16	76	x	x	x	x
RS16	46	RS16	80	x	x	x	x
RS16	50	RS16	80			x	x
RS16	46/50	RS4	185	x			x

RS16	46/50	RS16	80			x	
RS16	12/13	RS16	46/50			x	
RS16	12/13	RS16	46		x		
RS17	30	RS8	87	x	x	x	x
RS17	39	RS15	71	x	x	x	x
RS17	16/19	RS15	71	x	x	x	x
RS17	16	RS15	71	x	x	x	x
RS17	16/19	RS17	41/42		x	x	x
RS17	30	RS17	39	x		x	x
RS17	39	RS15	73				x
RS17	16/19	RS8	89			x	
RS17	16/19	RS17	41/42/43		x		
RS17	16/19	RS15	69/71	x			
RS18	9	RS21	22/25	x	x	x	x
RS18	9	RS21	25	x	x	x	x
RS18	9	RS18	36/38	x	x		x
RS18	9	RS21	59	x	x		x
RS18	38	RS6	106	x	x	x	
RS18	28	RS18	38		x	x	
RS18	36/38	RS6	106		x	x	
RS18	9	RS21	54		x	x	
RS18	9	RS21	58		x	x	
RS18	9	RS18	50	x			x
RS18	9	RS1	162	x	x		
RS18	9	RS6	35				x
RS18	9	RS18	14/23/24			x	
RS18	9	RS18	38			x	
RS18	30	RS6	104/106		x		
RS18	30/32	RS6	56		x		
RS18	9	RS6	92/93		x		
RS18	50	RS6	92		x		
RS18	9	RS1	158/162		x		
RS18	30/32	RS6	104/106		x		
RS18	9	RS6	106	x			
RS18	38	RS6	104/106	x			
RS18	9	RS21	22	x			
RS18	50	RS6	92/93	x			
RS18	36/38	RS1	158	x			
RS18	38	RS1	162	x			
RS18	36/38	RS1	247	x			
RS18	9	RS6	104/106	x			
RS18	9	RS1	158	x			

RS18	36/38	RS6	104/106	x			
RS18 and RL3 @56-59	56	RL3	62		x	x	x
RS18 and RL3 @56-59	56	RL3	61/62				x
RS18 and RL3 @56-59	56	RL3	61/62/70		x		
RS19	6/7	RL31	62	x	x	x	x
RS19	17/18	RL31	69/70	x	x	x	x
RS19	17/18	RS14	50		x	x	x
RS19	17/18	RS14	23	x		x	x
RS19	17/18	RL31	62	x	x	x	
RS19	29	RL5	120/121	x	x	x	
RS19	17/18	RS19	25/28	x	x	x	
RS19	28/29	RL31	69/70	x	x		
RS19	28/29	RL5	120/121	x	x		
RS19	17/18	RS19	21	x	x		
RS19	87/88	RS13	117/118	x	x		
RS19	17/18	RS14	47/50				x
RS19	0/4	RL5	120			x	
RS19	25/28/29	RL5	120/121		x		
RS19	28/29	RL31	62	x			
RS19	18	RS19	29	x			
RS19	25/28	RL31	69/70	x			
RS19	17/18	RS19	25	x			
RS19	28	RL5	120	x			
RS19	29	RL5	120	x			
RS2	28	RS2	45	x	x	x	x
RS2	112	RS3	147				x
RS2	112	RS3	147/150			x	
RS2	28	RS8	29/30/31			x	
RS2	130	RS3	233			x	
RS2	45	RL27 and RL10 @106-109	109			x	
RS2	128/130/131	RS3	147		x		
RS2	66	RS3	135	x			
RS2	105/106	RS3	135	x			
RS2	72/73	RS2	81	x			
RS2	66	RL15	128/129	x			
RS2	11	RS1	14	x			
RS2	81	RS3	108	x			
RS2	37	RS21	59	x			

RS2	37	RS4 and RS1 @87-90	90	x			
RS2	128	RS3	147	x			
RS2	66	RS2	115	x			
RS20	19	RL19	104/106	x	x	x	x
RS20	16	RS20	19	x	x	x	x
RS20	19	RL2	259		x	x	x
RS20	8/9	RS20	16		x	x	x
RS20	8/9	RS20	19		x	x	x
RS20	34	RS20	49	x	x	x	
RS20	49	RS20	85	x	x	x	
RS20	33/34	RS20	49	x		x	
RS20	33/34	RS20	71	x	x		
RS20	16	RS20	23				x
RS20	16	RS20	19/23				x
RS20	33/34	RS20	85			x	
RS20	44	RS20	76		x		
RS20	44	RS20	85		x		
RS21	54	RS21	59	x	x	x	x
RS21	25	RS18	30	x		x	x
RS21	25	RS18	38	x	x		x
RS21	59	RS9	129	x	x	x	
RS21	54	RS9	129	x		x	
RS21	49	RS21	59	x	x		
RS21	54	RS1	117	x	x		
RS21	59	RL10	150			x	
RS21	54	RS21	58			x	
RS21	49	RS21	58		x		
RS21	59	RS4	150		x		
RS21	25	RS18	36/38		x		
RS21	25	RL22	81/83		x		
RS21	43	RL14	67	x			
RS21	25	RS18	42	x			
RS3	108	RS3	147	x	x		x
RS3	79/80	RS3	86	x	x		
RS3	147	RS3	226	x	x		
RS3	147	RS3	233	x			
RS3	135	RS1	464	x			
RS3	233	RS1	279/281	x			
RS3	16	RS3	108	x			
RS3	150	RS3	226	x			
RS3	62/63	RS1	555	x			

RS4	167	RS4	183	x	x	x	x
RS4	151	RS4	156	x	x	x	x
RS4	83	RS5	167	x	x	x	x
RS4	83	RS4	185		x	x	x
RS4	156	RS4	167	x	x		x
RS4	151	RS4	181/183	x	x	x	
RS4	86	RS4	185			x	x
RS4	8/10	RS12	44			x	x
RS4	77	RS4	205/206			x	x
RS4	151/153	RS4	156		x	x	
RS4	156	RS4	177	x	x		
RS4	156	RS4	181/183	x	x		
RS4	8/10	RS8	74				x
RS4	10	RS4	22/23				x
RS4	77	RS4	83				x
RS4	22	RL28	52/54			x	
RS4	10/12	RS12	44			x	
RS4	22/23	RS12	44			x	
RS4	10	RS12	44		x		
RS4	151	RS4	177	x			
RS4	156	RS4	177/181/183	x			
RS5	66	RL7	109	x	x		
RS5	26	RS9	129	x	x		
RS5	26	RS3	135	x	x		
RS5	26	RS5	52				x
RS5	66	RS3	135				x
RS5	62	RS3	89			x	
RS5	86	RS8	87		x		
RS5	52	RS5	66		x		
RS5	23/24/26	RS5	52	x			
RS6	35	RS1	229	x			
RS7	11	RS9	100	x	x	x	x
RS7	76/77	RS7	131/133/136		x		x
RS7	76	RS7	136	x			x
RS7	114/115	RS7	131/133/136	x	x		
RS7	54/56	RS7	136	x	x		
RS7	17	RS9	105				x
RS7	11	RS7	20				x
RS7	11	RS7	25		x		
RS7	114	RS7	137		x		
RS7	76	RS1	260		x		
RS7	76	RS7	131/133/136		x		

RS7	72	RS7	136		x		
RS7	76	RS1	256/260	x			
RS7	114	RS7	136/137	x			
RS7	114/115	RS7	136	x			
RS7	54/56	RS1	247	x			
RS7	54/56	RS1	411	x			
RS7	11	RS1	247	x			
RS7	72	RS7	131	x			
RS8	89	RS5	167	x	x	x	x
RS8	31/33	RS8	50	x	x	x	x
RS8	94	RS5	167	x	x	x	x
RS8	22	RS2	28	x	x	x	
RS8	89/94	RS5	167	x	x	x	
RS8	50	RS8	69	x	x	x	
RS8	87	RS8	89	x	x	x	
RS8	108/112	RS5	167	x	x		
RS8	108	RS5	167	x	x		
RS8	89	RL1	186				x
RS8	87	RS5	92				x
RS8	30	RS8	50			x	
RS8	89	RS8	112		x		
RS8	33	RS8	50	x			
RS8	89	RS8	108	x			
RS8	89	RS8	108/112	x			
RS8	112	RS5	167	x			
RS9	100	RS9	115		x	x	x
RS9	100	RS9	114			x	x
RS9	100	RS1	278/279/281			x	x
RS9	115	RS9	128/129		x		x
RS9	100	RS1	411	x	x		
RS9	100	RS1	279			x	
RS9	100	RS9	120		x		
RS9	13	RS9	100		x		
RS9	100	RS1	247		x		
RS9	100	RS9	128	x			
RS9	100	RS1	278	x			
RS9	100	RS1	256	x			
RS9	100	RS1	279/281	x			