Supporting information: Studying protein-ligand interactions by protein-denaturation and quantitative cross-linking mass spectrometry

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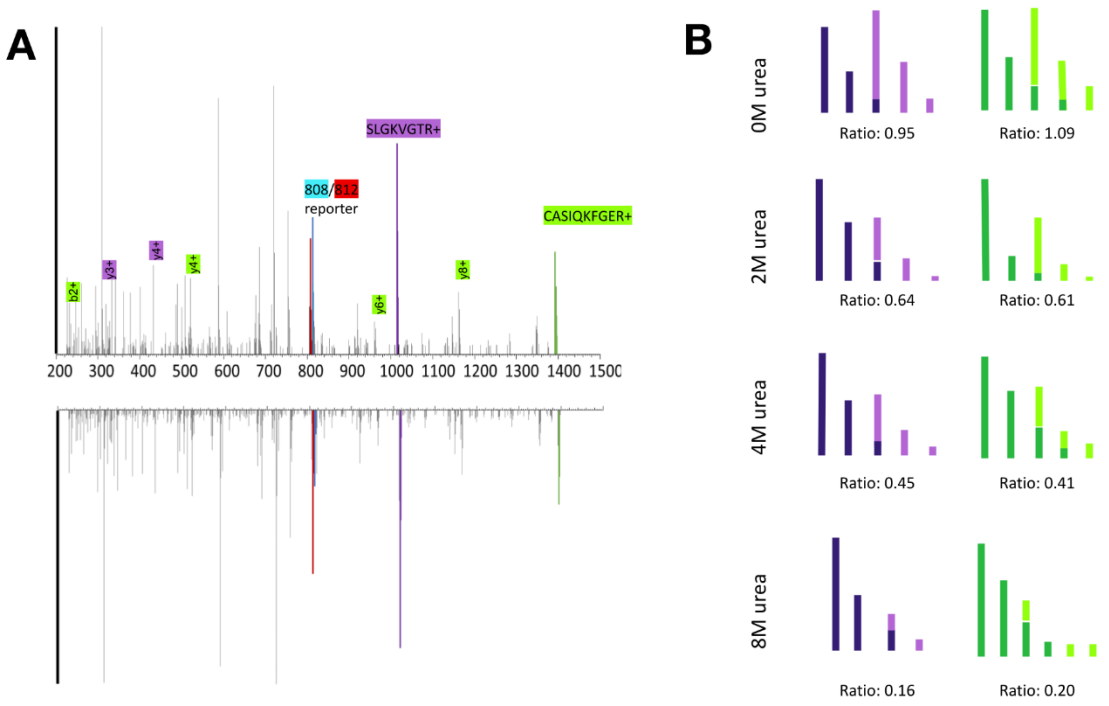
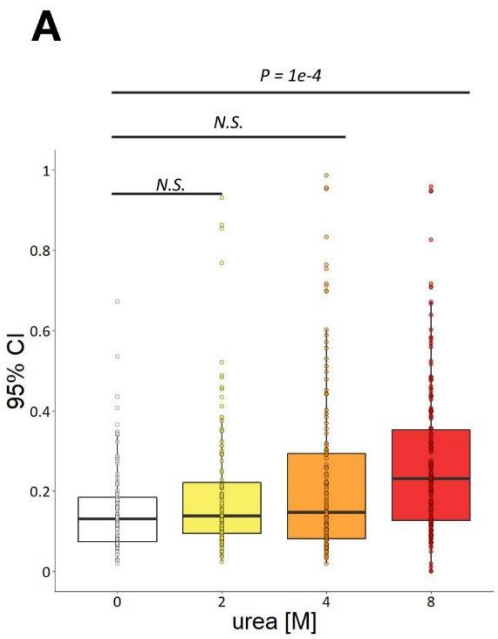
Table S1 Intrinsic fluorescence raw values

		BSA				BSA + Bilirubin			
wavelength (nm)	urea (M)	read1	read2	read3	read4	read1	read2	read3	read4
285,340	blank	905542	929100	941877	954277	964577	974777	978515	982012
	0M	1872350	1913862	1917623	1943602	935371	953342	959569	957943
	1M	1848377	1901654	1911590	1912605	960342	970801	957962	968613
	2M	1853717	1887760	1900256	1909050	980559	968915	986866	986548
	4M	1623144	1660588	1636913	1609713	1045383	1046835	1035523	1036251
	5M	1443980	1469245	1486383	1484604	1086800	1088126	1076233	1081879
	6M	1367241	1382496	1384268	1383285	1125213	1124910	1113191	1103956
	8M	1171097	1189576	1194937	1198420	1056541	1064910	1057553	1049156
488,530	blank	2414	2480	2606	2547	2578	2661	2674	2628
	0M	1826	1895	1903	1877	29072	29807	30167	30195
	1M	1838	1953	1966	1934	30792	31623	31678	31636
	2M	1879	2013	1965	1941	32797	33626	33395	33256
	4M	2032	2098	2064	2032	34756	34375	34129	33618
	5M	1992	2050	2068	2093	26903	27110	26886	27019
	6M	2034	2078	2129	2132	19219	19153	18954	18819
	8M	1950	1986	2077	2029	6123	6106	6098	6104



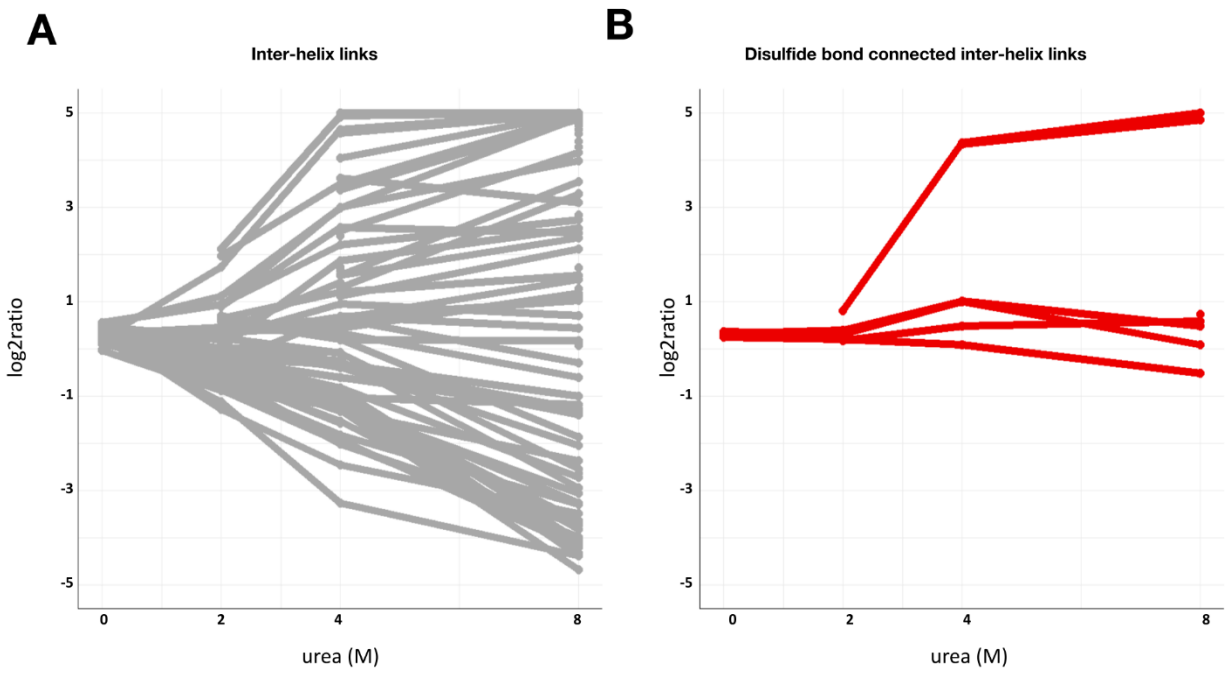
Supplemental Figure S1 BSA crosslink spectra between reciprocally labeled experimental replicates.

Example fragmentation and quantification spectrum of a BSA cross-linked peptide pair between reciprocally labeled experimental replicates. (A) Spectra of crosslink K228-K455 (CASIQK^{228}FGER- SLGK^{455}VGTR) in reciprocally labeled experiment 1 (top) and experiment 2 (bottom) displayed as mirror images. Peaks for reporter ions (iqPIR-808, red; iqPIR-812, blue), peptide CASIQK^{228}FGER (green), and peptide SLGK^{455}VGTR (purple), are highlighted. (B) Expanded views of released cross-linked peptides fragment ions illustrating the isotopic differences which are used for quantification. The resultant fragment ions differ by two 13C and deconvoluted signal from iqPIR-812-reporter (darker) and iqPIR-808 (urea-exposed, lighter) are shown. Example released peptide signals come from 0M urea (experiment 2, injection 1, scan 7043), 2M (experiment 2, injection 1, scan 7112), 4M (experiment 1, injection 1, scan 6990), 8M (experiment 1, injection 1, scan 6895).



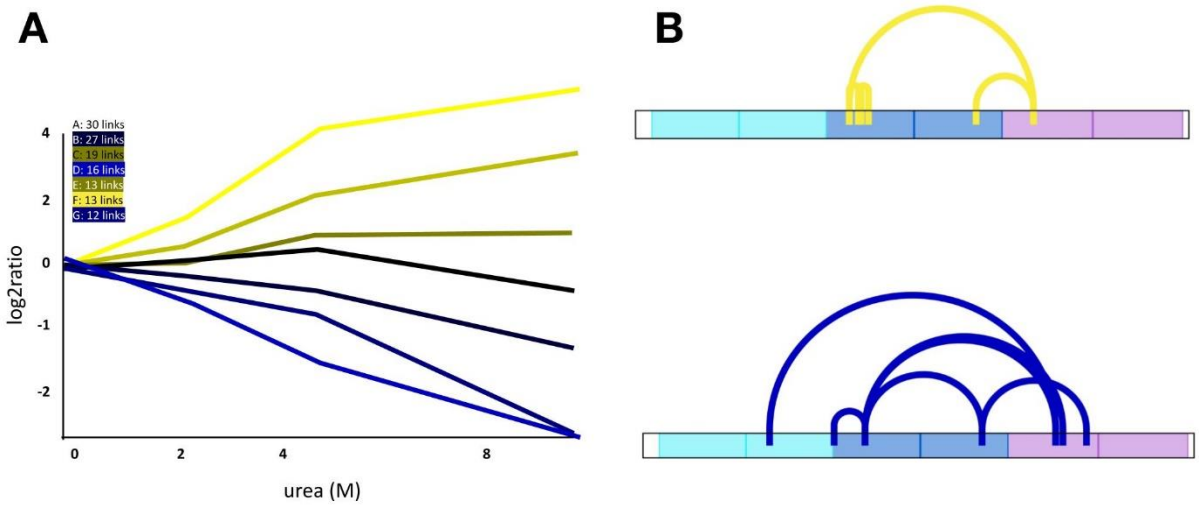
Supplemental Figure S2 Quantitation confidence does not decrease with urea exposure.

The distribution of associated 95% confidence intervals for quantified cross-links (Fig 2B) were plotted with urea exposure. Errors were compared by paired Student's t-test with Bonferroni multiple test correction (N.S. = not significant, ***P<0.01). The median confidence interval between 0M and 8M urea exposure was 0.12 (B)



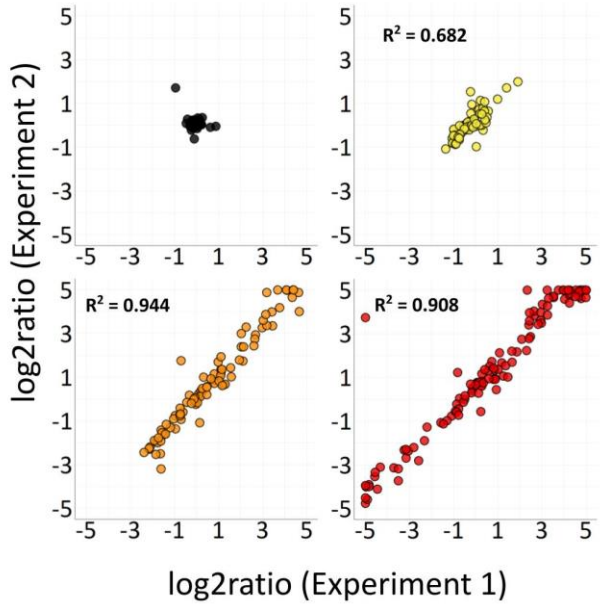
Supplemental Figure S3. qXL-MS data obtained on cross-link peptide pairs of lysines on disulfide linked helices.

(A) Urea concentration-dependent trends in cross-linked peptide pairs are identified by statistical filtering (3 of 4 concentrations quantified within a 95% confidence interval less than 1). (B) The average log2 values of cross-linked species mapped to helices connected by disulfide bonds are highlighted in red.



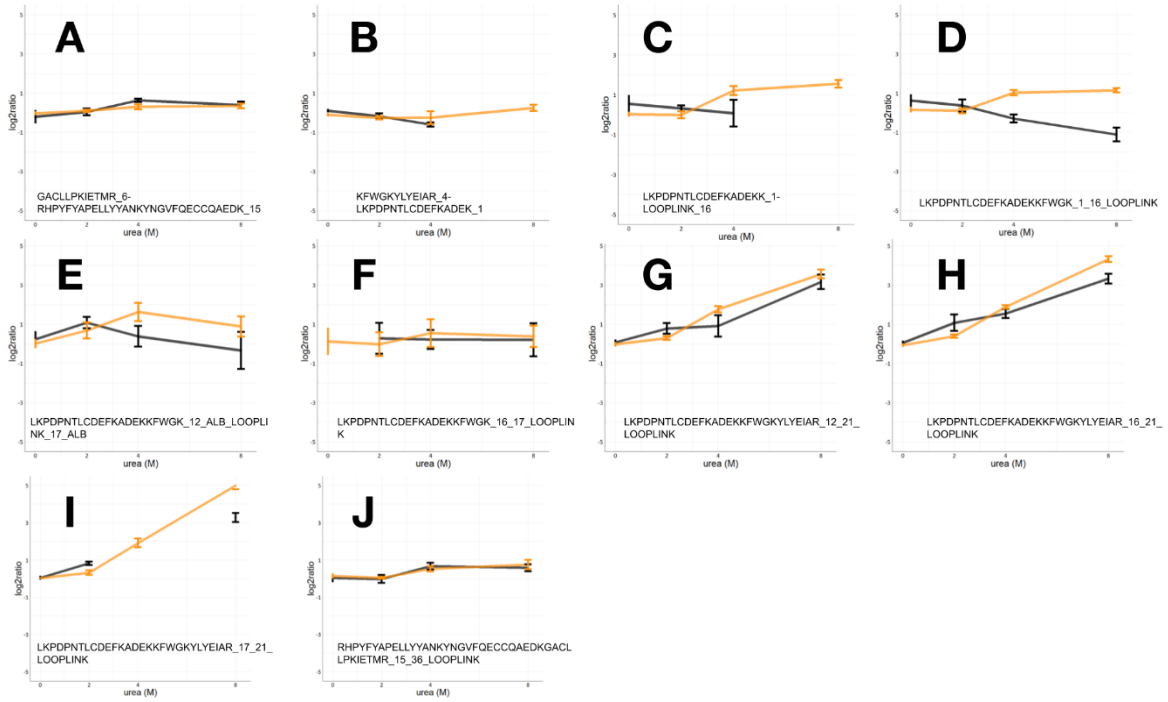
Supplemental Figure S4. K-means analysis of BSA urea structural stability by MS2-based quantitative cross-linking.

Urea concentration-dependent trends in cross-linked peptide pairs are identified by statistical filtering (3 of 4 concentrations quantified within a 95% confidence interval less than 1) and longitudinal k-means clustering. The average log2 values and number of cross-linked species present in each cluster are given. (B) Domain level cross-link maps of clusters that showed the largest changes, generated with XiNet, illustrate inter- and intra- domain differences between the clusters.



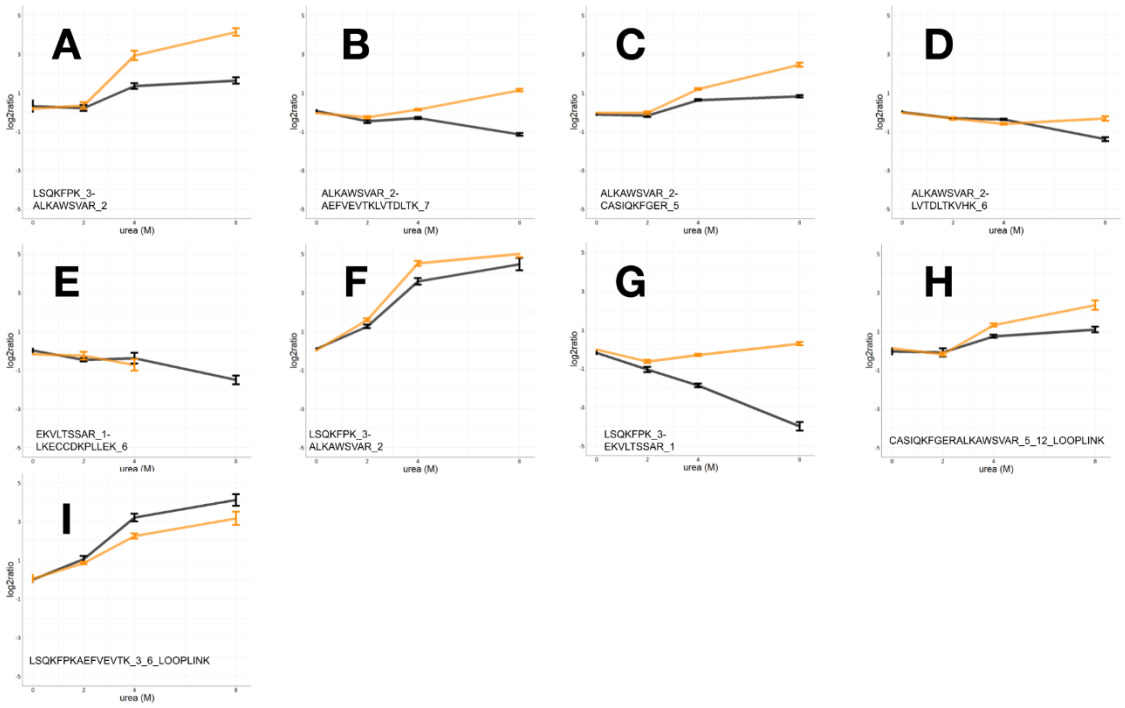
Supplemental Figure S5. Reproducibility of reciprocal labeling in BSA-bilirubin experiments.

Correlation of crosslink quantitation between two experimental replicates shown at 0M (black), 2M (yellow), 4M (orange), and 8M (red) urea exposure. Pearson's R2 values are given for each condition, indicating the reproducible observations of urea-induced unfolding and qXL-MS measurements. Cross-link quantification is available on XLinkDB as dataset inUrea_BSA_wBR_fwd_rev_Bruce.



Supplemental Figure S6. qXL-MS data obtained on cross-link peptide pairs of lysine residues in domain IB.

(A-I) Shown are the relative log2ratios obtained for each peptide pairs at increasing urea concentrations for BSA (black) or BSA-bilirubin (orange). Error bars represent 95% quantitation confidence given all relevant calculated MS2-based ion pairs across experimental duplicate and technical triplicate. While many cross-linked peptide level changes are insensitive or unaltered by bilirubin interaction, cross-linked peptide K140-K155 (D) how increased urea-induced level changes in the presence of bilirubin. Thus, bilirubin binding with BSA appears to affect protein stability within the IB domain as indicated by qXL-MS.



Supplemental Figure S7. qXLMS data obtained on cross-link peptide pairs of lysines residues in domain IIA.

(A-J) Shown are the relative log2ratios obtained for each peptide pairs at increasing urea concentrations for BSA (black) or BSA-bilirubin (orange). Error bars represent 95% quantitation confidence given all relevant calculated MS2-based ion pairs across experimental duplicate and technical triplicate.