

Fig. S1: The full m/z ratios, 700-1800, of CstR (A-C) and CsoR (D-F). A and D are unreacted controls, B and E were reacted with SeO_3^{2-} , and C and F reacted with TeO_3^{2-} as described in the materials and methods. Unreacted CstR displays a m/z range from +6 to +13 and cross-linked CstR ranges from +11 to +20. Unreacted CsoR displays a m/z range from +7 to +15 and cross-linked CsoR from +13 to +23.

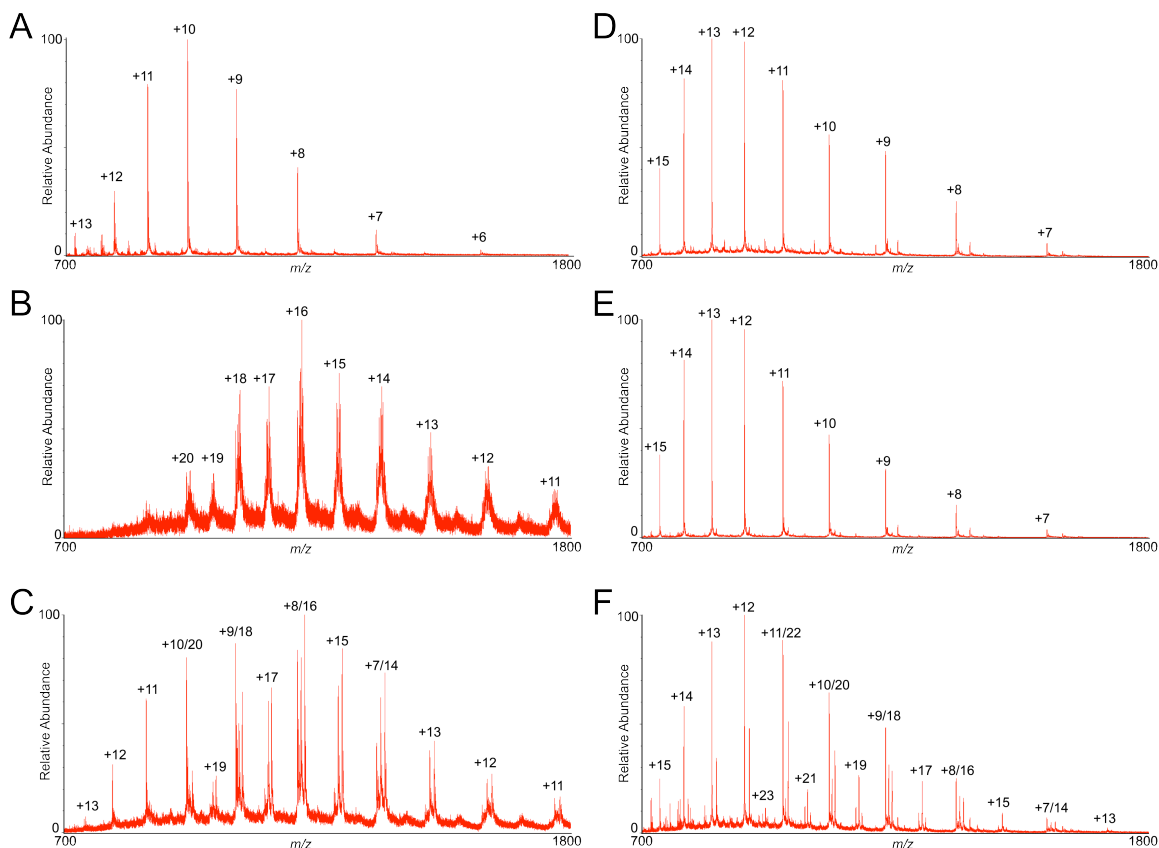


Fig. S2: Fragmentation patterns of the Cys31 (A), MMEEGKDCKDVITQISASK, and Cys60 (B), LMGIISENLIECVK, containing peptides from a tryptic digest of reduced CstR. Both peptides readily fragment as most ions are readily observed. These spectra were used to help identify cross-linked CstR peptides.

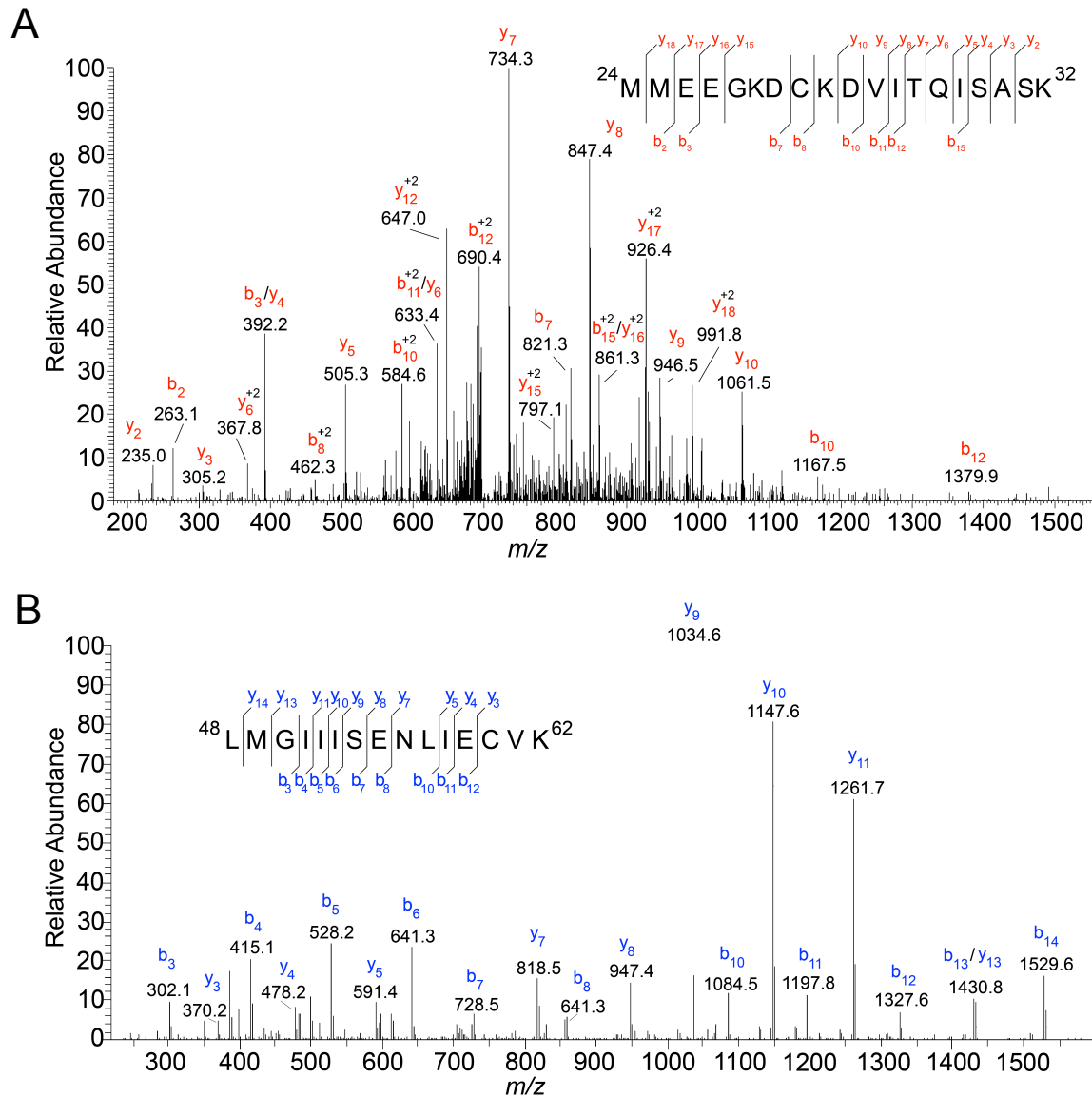


Fig. S3: CstR reacted with SeO_3^{2-} contains prominent disulfide peaks with oxidized cysteine residues. (A) Sum of +4 ions detected over the elution range of CstR cross-linked species. The labeled cross-linked peptides are; **1**, disulfide cross-linked CstR (fragmentation pattern in Fig. 2B, main text); **2**, disulfide cross-linked CstR with one oxidized methionine (fragmentation data not shown); **3**, disulfide cross-linked CstR with two oxygen atoms distributed between the cysteine residues (fragmentation shown in panel B); **4**, Same as **3** with an oxidized methionine (fragmentation data not shown); **5**, selenotrisulfide cross-linked CstR (fragmentation pattern in Fig. 3D, main text); **6**, selenotrisulfide cross-linked CstR with one oxidized methionine (fragmentation data not shown). (B) Fragmentation pattern of disulfide cross-linked CstR with two cysteine oxidations (peak **3** from panel A). Cross-linked AB_{yn} ions contain a mass shift of +32 Da and correspond to two total oxygen atom adductions on one or both cysteine S_γ atom. An oxidation of either Met residue on peptide “A” yields an $\text{A}_{\text{b}7}$ ion with a mass of approximately 837 or 853 Da for one or two oxygen atoms, respectively. The observed $\text{A}_{\text{b}7}$ ion has a mass consistent with the reduced state, 821 Da. Similarly, a Met residue oxidation on peptide “B” would yield B_{bn} ions with a mass shift of +16 or +32 Da, e.g., the observed $\text{B}_{\text{b}3}$ ion would shift to approximately 318 or 334 Da with one or two oxygen atoms, respectively. As a result, we conclude that the oxygen adducts here (*) are assignable as either a mixture of thiosulfonates or α -disulfoxide (Fig. 4, main text).

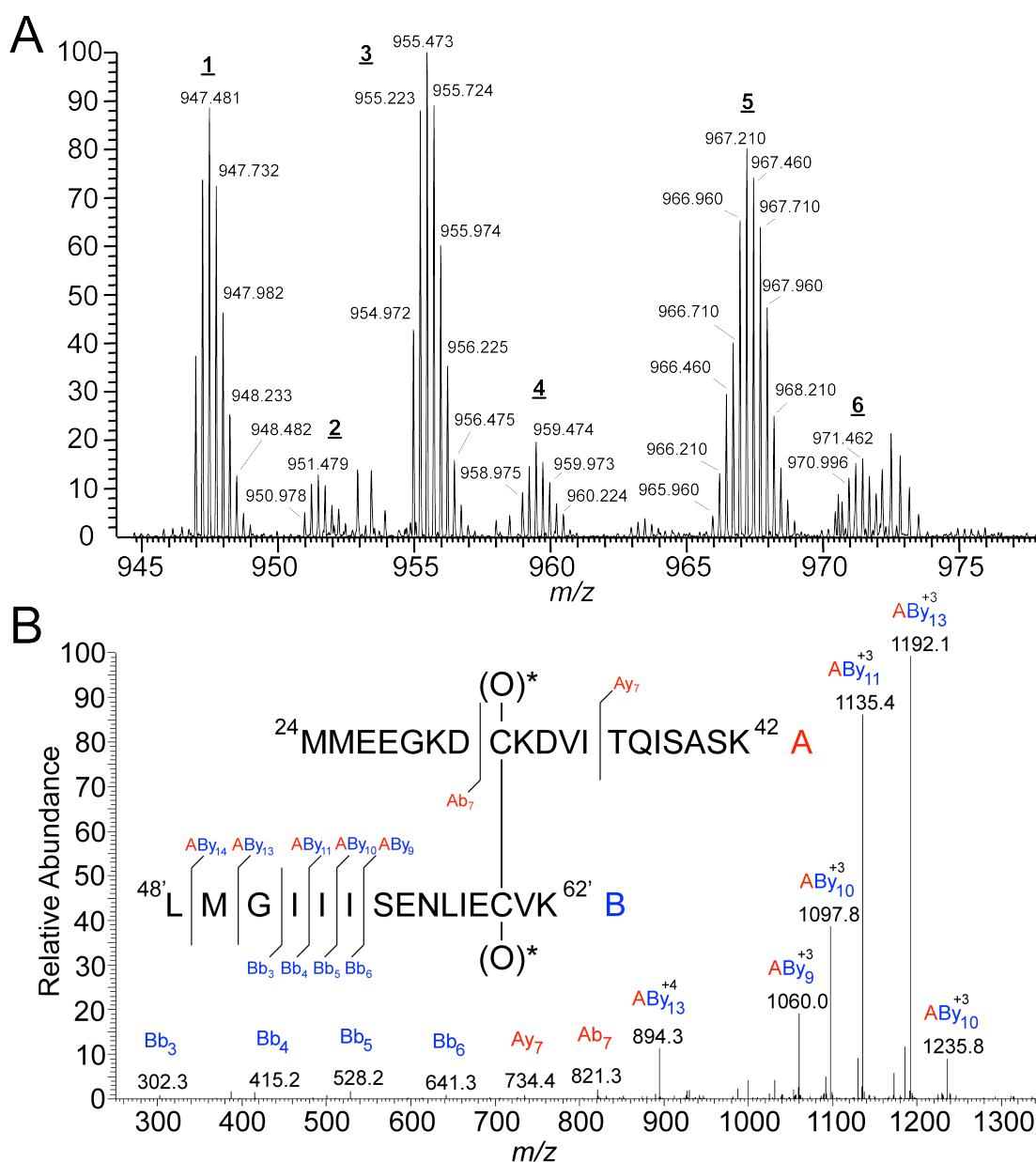


Fig. S4: Reaction products observed by ESI-MS from the reaction (17 h, RT) of C31A CstR (A and B) and C60A CstR (C and D) with tetrathionate (panels A and C) and SeO_3^{2-} (panels B and D). Deconvoluted masses from these spectra are shown in Table S4. Addition of tetrathionate to C31A CstR leads to the formation of disulfide cross-linked CstR as $^{60}\text{CysS-SCys}^{60}$ likely across the tetramer interface (see Fig. 1, main text) (m/z of +12 to +24). The analogous reaction with C60A CstR forms nearly exclusively $^{31}\text{CysS-S}_2\text{O}_3$ (m/z of +7 to +12). Reaction with selenite (panels B and D) leads to significant unreacted, reduced CstRs and a mixture of products dominated by the di- and selenotrisulfide cross-linked species, again likely linking cysteines on different dimers. In addition to these species, the reaction of C60A CstR with selenite (panel D) also yields a detectable amount of the monofunctionalized $^{31}\text{CysS-SeO}_3^{2-}$ (filled circles) (Table S4). The small amount of this product is consistent with that of a reaction intermediate on pathways to cross-linked species, although this was not investigated here. (E) Fluorescence anisotropy titrations of reduced C31A (open circles*) or C60A (open squares*) CstR before and following full derivatization with selenite (closed symbols). Derivatization was complete after 48 h. *Titrations shown are the same as those shown in Fig. 5, main text.

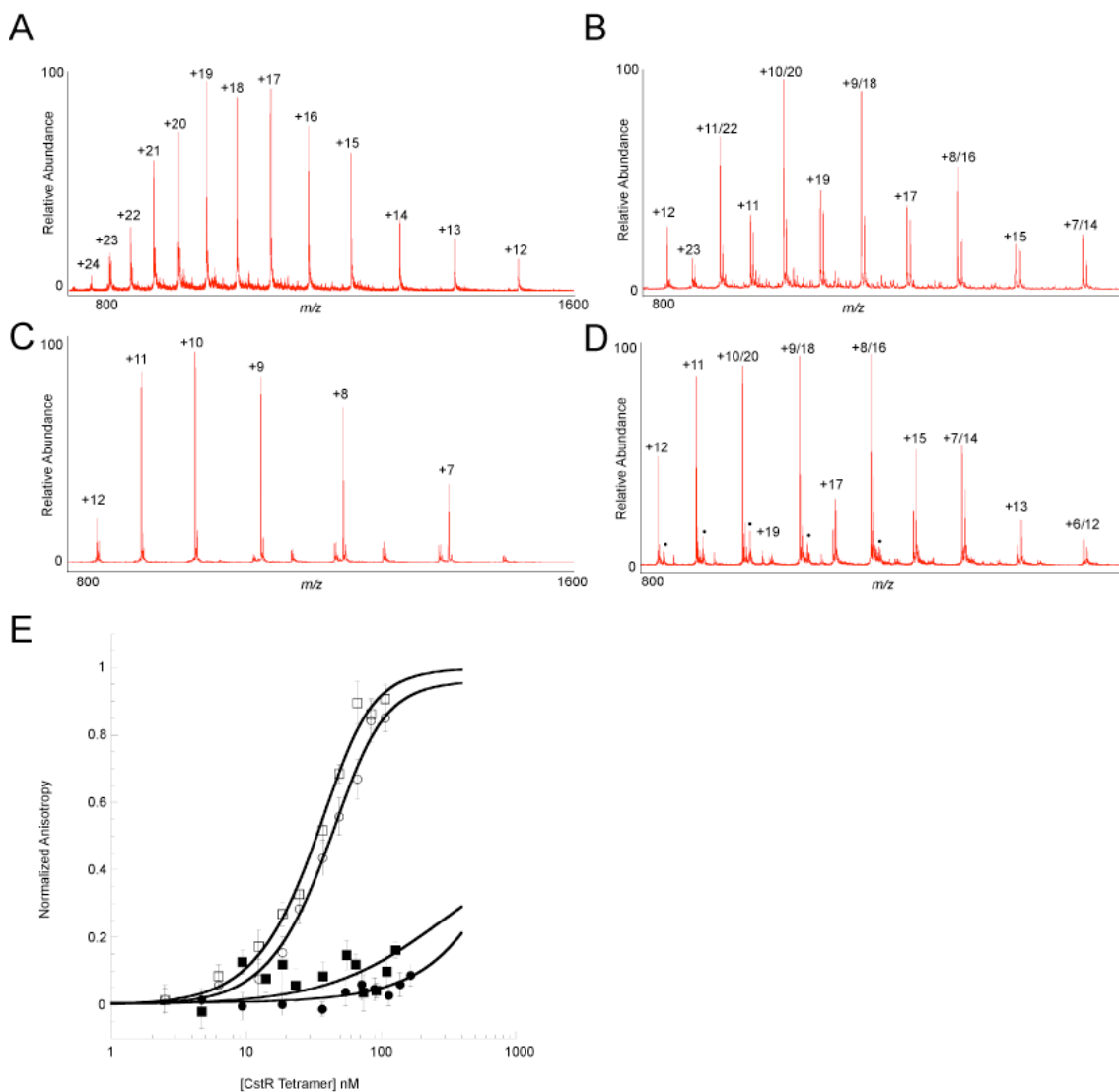


Table S1: CstR cysteine mutant Quikchange™ mutagenesis primers used in this study. Underlined characters are bases changed to introduce cysteine to alanine mutations. *cst* OP1 corresponds to the DNA used in fluorescence anisotropy experiments where ‘F’ denotes fluorescein.

Primer	Direction	Primer Sequence
C31A CstR	Forward	GGAGGAAGGAAAAGAC <u>GCT</u> AAGATGTCATTAC
	Reverse	GTAATGACATCTTTAG <u>CGT</u> CTTTTCCTTCCTCC
C60A CstR	Forward	GTGAGAATTTAATAGAA <u>GCT</u> GTAAGAGCAGCTGCGG
	Reverse	CCGCAGCTGCTTTTACAGCTTCTATTAAATTCTCAC
<i>cst</i> OP 1	Forward	ATGTGTCAAATACCCCTAGAGGTATTTG
	Reverse	F-CAAATACCTCTAGGGGTATTTGACACAT

Table S2: Summary of deconvoluted CstR masses observed by LC-ESI-MS. All mass shifts are relative to CstR^{RS-H} or CstR₂^(RS-SR') as indicated by (-).

Modifier	M _r Expected	M _r Observed	Mass Shift	Assignment
None	9641.2	9641.3	-	CstR ^{RS-H}
	9663.2	6443.4	22.1	CstR ^{RS-H} + Na
	19280.4	19282.3	-	CstR ₂ ^{RS-SR'}
SeO ₃ ²⁻	9641.2	9638.2	-	CstR ^{RS-H}
	9663.2	9660.4	22.2	CstR ^{RS-H} + Na
	19280.4	19277.5	-	CstR ₂ ^{RS-SR'}
	19312.4	19309.7	32.2	CstR ₂ ^{RS-SR'} + 2 O
	19359.4	19357.4	79.9	CstR ₂ ^{RS-Se-SR'}
	19391.4	19389.6	112.1	CstR ₂ ^{RS-Se-SR'} + 2 O
	19425.4	19422.5	145	CstR ₂ ^{RS-Se-SR'} + 3 Na
	19438.3	19437.1	159.6	CstR ₂ ^{(RS-Se-SR')2}
	19470.3	19468.8	191.3	CstR ₂ ^{(RS-Se-SR')2} + 2 O
	19503.4	19500.3	222.8	CstR ₂ ^{(RS-Se-SR')2} + 3 Na
TeO ₃ ²⁻	9641.2	9640.2	-	CstR ^{RS-H}
	9657.2	9656.2	16	CstR ^{RS-H} + O
	9673.2	9671.6	31.4	CstR ^{RS-H} + 2 O
	9768.8	9767.5	127.3	CstR ^{RS-H} + Te
	19280.4	19279.7	-	CstR ₂ ^{RS-SR'}
	19312.4	19311.4	31.7	CstR ₂ ^{RS-SR'} + 2 O
	19408	19407.3	127.6	CstR ₂ ^{RS-Te-SR'}
	19440	19439.1	159.4	CstR ₂ ^{RS-Te-SR'} + 2 O
	19535.6	19534.6	254.9	CstR ₂ ^{(RS-Te-SR')2}
	19567.6	19567.6	287.9	CstR ₂ ^{(RS-Te-SR')2} + 2 O
S ₄ O ₆ ²⁻	9641.2	-	-	CstR ^{RS-H}
	19280.4	19279.4	-	CstR ₂ ^{RS-SR'}
	19302.4	19300.6	21.2	CstR ₂ ^{RS-SR'} + Na
	19312.4	19311.6	32.2	CstR ₂ ^{RS-S-SR'}
	19324.4	19322.4	43	CstR ₂ ^{RS-SR'} + 2 Na

Table S3: Summary of deconvoluted CsoR masses observed by LC-ESI-MS. All mass shifts are relative to CsoR^{RS-H} or CsoR₂^(RS-SR') as indicated by (-).

Modifier	M _r Expected	M _r Observed	Mass Shift	Assignment
None	11036.6	11034.9	-	CsoR ^{RS-H}
	11058.6	11057.0	22.1	CsoR ^{RS-H} + Na
SeO ₃ ²⁻	11036.6	11035.4	-	CsoR ^{RS-H}
	11058.6	11057.5	22.1	CsoR ^{RS-H} + Na
TeO ₃ ²⁻	11036.6	11033.2	-	CsoR ^{RS-H}
	21069.2	22065.4	-	CsoR ^{RS-SR'}
	21196.8	22192.2	126.8	CsoR ₂ ^{RS-Te-SR'}
	21324.4	22318.1	252.7	CsoR ₂ ^{(RS-Te-SR')2}

Table S4: Summary of deconvoluted CstR cysteine mutant masses observed by LC-ESI-MS. All mass shifts are relative to CstR^{RS-H} or CstR₂^{RS-SR'} as indicated by (-).*

Protein	Modifier	M _r Expected	M _r Observed	Mass Shift	Assignment		
C31A	None	9609.1	9607.2	-	CstR ^{RS-H}		
	MMTS	9656.2	9653.3	46.1	CstR ^{RS-SCH3}		
	SO ₃ ²⁻	9609.1	9606.5	-	CstR ^{RS-H}		
		9631.1	9627.9	21.4	CstR ^{RS-H} + Na		
		9653.1	9648.6	42.1	CstR ^{RS-H} + 2 Na		
		SeO ₃ ²⁻	9609.1	9606.0	-	CstR ^{RS-H}	
		9625.1	9622.3	16.3	CstR ^{RS-H} + O		
		19216.2	19211.6	-	CstR ₂ ^{RS-SR'}		
		19295.2	19290.2	78.6	CstR ₂ ^{RS-Se-SR'}		
		19327.2	19326.4	114.8	CstR ₂ ^{RS-Se-SR'} + 2 O		
	S ₄ O ₆ ²⁻	9609.1	9606.2	-	CstR ^{RS-H}		
		19216.2	19211.5	-	CstR ₂ ^{RS-SR'}		
		19232.2	19227.5	16.0	CstR ₂ ^{RS-SR'} + O		
		19238.2	19232.6	21.1	CstR ₂ ^{RS-SR'} + 2 Na		
		19260.2	19254.8	43.3	CstR ₂ ^{RS-SR'} + 2 Na		
		C60A	None	9609.1	9607.0	-	CstR ^{RS-H}
			MMTS	9656.2	9653.5	46.5	CstR ^{RS-SCH3}
			SO ₃ ²⁻	9609.1	9607.3	-	CstR ^{RS-H}
	9631.1			9628.3	21.0	CstR ^{RS-H} + Na	
	9689.2			9687.4	80.1	CstR ^{RS-SO3}	
9711.2	9708.3			101.0	CstR ^{RS-SO3} + Na		
SeO ₃ ²⁻	9609.1		9606	-	CstR ^{RS-H}		
	9625.1		9622.5	16.5	CstR ^{RS-H} + O		
	9641.1		9638.3	32.3	CstR ^{RS-H} + 2O		
	9688.1		9684.5	78.5	CstR ^{RS-H} + Se		
	9720.1	9717.7	111.7	CstR ^{RS-SeO2}			
	9736.1	9738.8	132.8	CstR ^{RS-SeO3}			
	19216.2	19211.6	-	CstR ₂ ^{RS-SR'}			
	19248.2	19245.4	33.8	CstR ₂ ^{RS-SR'} + 2 O			
	19295.2	19290.3	78.7	CstR ₂ ^{RS-Se-SR'}			
	19327.2	19323.3	111.7	CstR ₂ ^{RS-Se-SR'} + 2 O			
S ₄ O ₆ ²⁻	9609.1	-	-	CstR ^{RS-H}			
	9721.1	9718.1	112.1	CstR ^{RS-S2O3}			
	9753.1	9750.7	144.7	CstR ^{RS-S3O3}			
	C31/60A	None	9577.0	9575.3	-	No Thiol	

*Major species observable by ESI-MS deconvoluted from the data shown in Fig. S4 are highlighted in red. The expected RS-SeO₃²⁻ adduct on Cys31 in C60A CstR is highlighted in bold font.