Supporting Information

Supplementary Figures

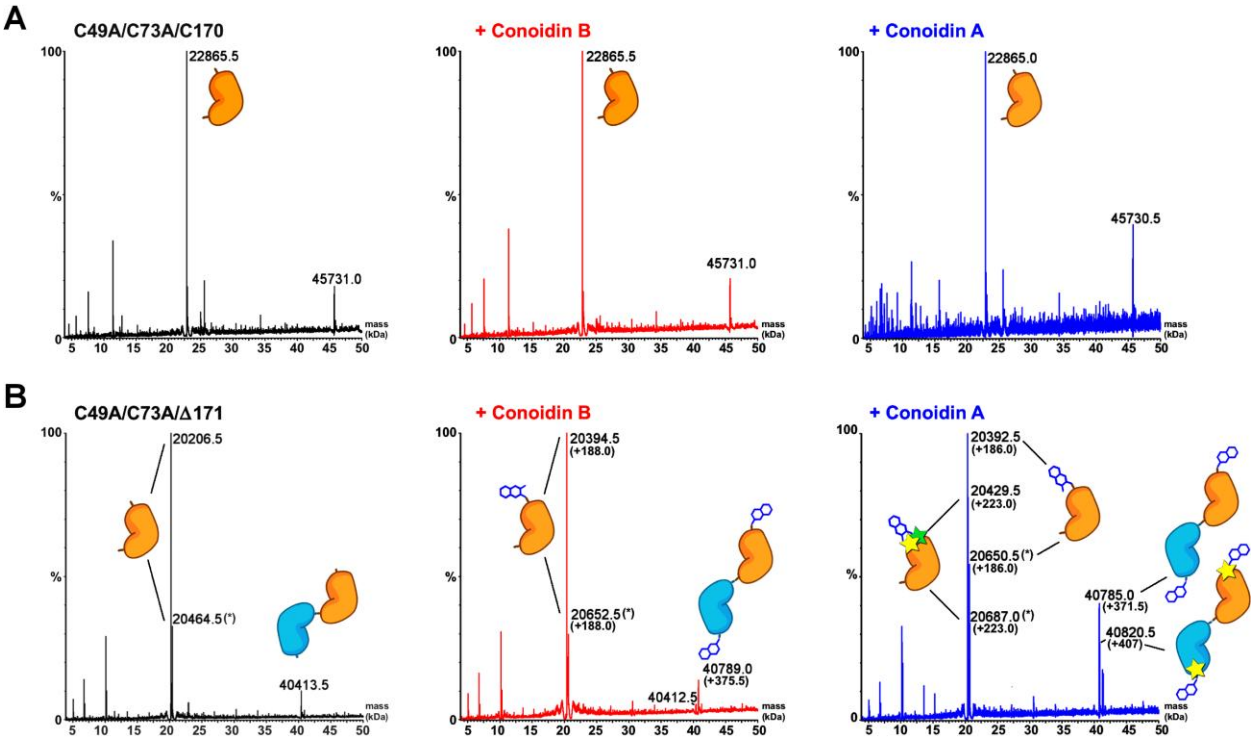

Figure S1, related to Figure 4. Conoidin A and conoidin B covalently modify mutant AcePrx-1 by alkylation and/or crosslinking based on electrospray mass spectra (LC-ESI-MS). Modified and unmodified proteins are indicated by schematic drawings. The quinoxaline dioxide (QDO) adduct formed upon reaction is represented as two conjoined hexagons. The filled hexagon indicates deoxygenation to the mono-oxide. Each star represents addition of a single oxygen to the protein.

A. The C49A/C73A/C170A triple mutant (left) does not produce a mass change in the presence of conoidin A (right) or conoidin B (middle), confirming that cysteine residues are the specific site of modification on AcePrx-1.

B. The C49A/C73A/Δ171 predominately forms monomeric alkylation products rather than dimeric crosslinks due to the sole presence of Cys170 to initiate nucleophilic S_N2 substitution.

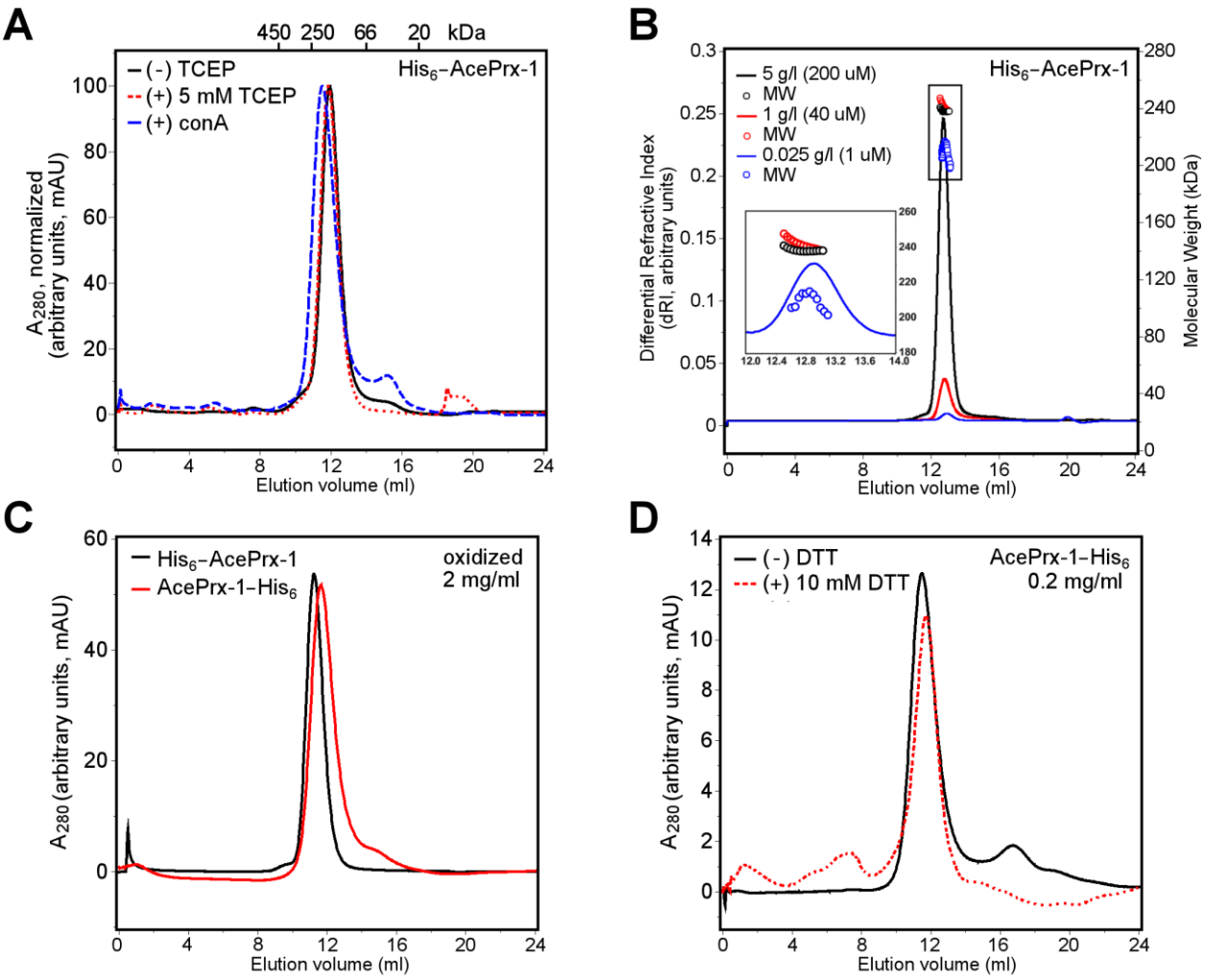

Figure S2, related to Figure 5. AcePrx-1 forms a stable decamer.

A. Size-exclusion chromatography in the presence and absence of conoidin A and reducing agent indicates that AcePrx-1 forms a large oligomer, which remains stable upon oxidation or crosslinking with conoidin A. The buffer was 10 mM HEPES 7.5, 100 mM NaCl.

B. Multi-angle light scattering (MALS) traces for AcePrx-1 under non-reducing conditions (10 mM HEPES 7.5, 100 mM NaCl) superimposed on the corresponding size-exclusion chromatography trace at different AcePrx-1 concentrations: 200 µM (black), 40 µM (red), and 1 µM (blue). AcePrx-1 is a stable decamer even in its oxidized form. The N-terminal 6xHis tag was not removed during purification.

C and D. Size-exclusion chromatographs of a C-terminal 6xHis tag construct (2 g/l or 0.2 g/l) suggest that it is a stable decamer in 10 mM HEPES 7.5, 100 mM NaCl, 5 mM EDTA, +/- 10 mM DTT as indicated.

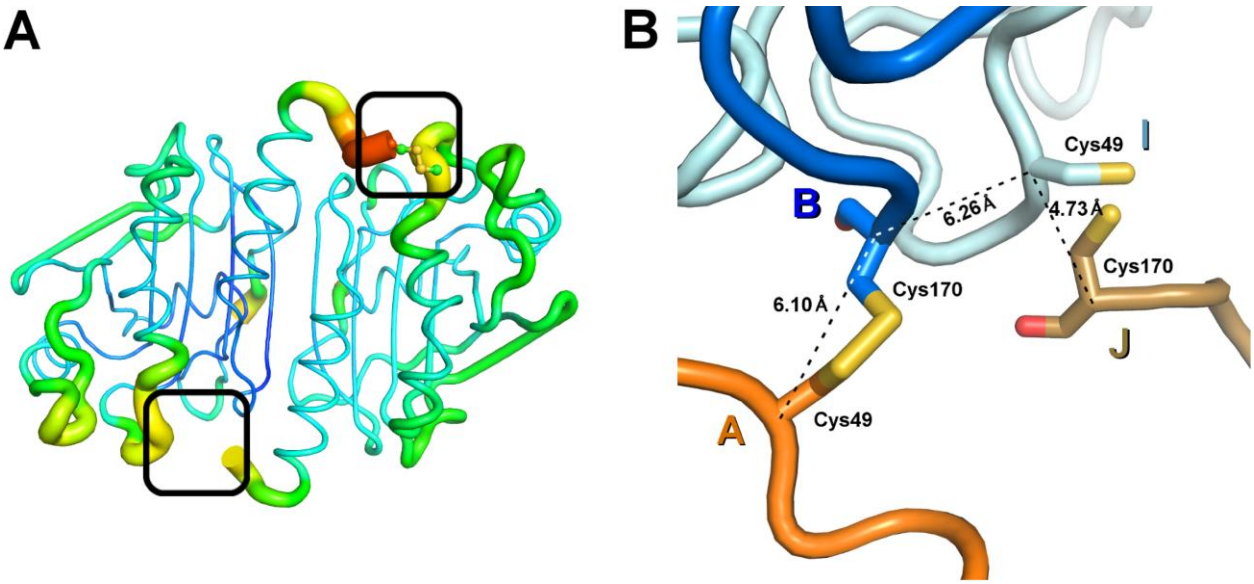

Figure S3, related to Figure 6. Disorder in the region of the catalytic cysteine residues. B-factor "putty" diagram showing increased conformational flexibility in the regions containing the active site cysteines in the structure of unliganded AcePrx-1.

Thin blue lines signify regions with low temperature factors (B-factors), which are indicative of low conformational flexibility and small thermal motions; thick red lines signify high B-factors, which are indicative of high conformational flexibility are large thermal motions.

Table S1, related to Figures 3 and 4. Detailed results from electrospray ionization mass spectrometry on WT and mutant AcePrx-1 in the presence and absence of conoidin A or conoidin B. Masses are listed in order of abundance.

Protein	Inhibitor	1mer mass	Mass diff	Modifications	2mer mass	Mass diff	Modifications
WT	--	22960.0			45920.0		
		23004.5(*)			46009.0(*)		
		23049.0(**)			46279.0(***)		
	conB	23337.5	377.5	2*QDO	46279.0	359.0	QDO + QMO
		23181.0	221.0	2*-SOH, QDO			
		23149.0	189.0	QDO			
		23488.0	528.0	QDO + 2*QMO (or 2*QDO + Qx)			
	conA	23147.0	187.0	QDO	46294.5	374.0	2*QDO
		23192.0(*)	187.5	QDO	46384.0(*)	375.0	2*QDO
		23237.0(**)	188.0	QDO	46473.0(**)	375.0	2*QDO
					46553.5(***)	374.5	2*QDO
C73A/C170A	--	22897.0			45794.0		
	conB	22897.0	0.0	--	45794.5	0.5	--
		23085.0	188.0	QDO	46170.5	376.5	2*QDO
		23067.0	170.0	QMO			
		23275.0	378.5	2*QDO			
	conA	23120.0	223.0	2*-SOH, QDO	46239.0	445.0	2*SOH, QDO
		22898.5	1.5	--	45793.5	-0.5	--
		23100.0	203.0	-SOH, QDO			
C49A/C73A	--	22897.5					
	conB	23085.5	188.0	QDO			
		23067.5	170.0	QMO			
		23105.0	207.5	-SOH, QDO			
	conA	23083.5	186.0	QDO			
		23121.0	223.5	-2*-SOH, QDO			
		23103.0	205.5	-SOH, QDO			
C49A/C170A	--	22897.5					
	conB	22898.0	0.5	--			
	conA	22898.0	0.0	--			
C49A/C73A/C170A	--	22865.5					

	conB	22865.5	0.0	--			
	conA	22865.0 23087.0[§]	-0.5 221.5[§]	-- 2*-SOH, QDO[§]			
Protein	**Inhibitor**	**1mer mass**	**Mass diff**	**Modifications**	**2mer mass**	**Mass diff**	**Modifications**
WTΔ171	--	20270.5			40540.0		
	conB	20647.0	376.5	2*QDO	40537.5	-2.5	--
		20629.0	360.0	QMO+QDO	41294.5	754.5	4*QDO
		20665.5	395.0	2*-SOH, QDO	41259.0	719.0	Qx+3*QDO
		20473.5	203.0	-SOH, QDO	40913.0	373.0	2*QDO
		20456.5	186.0	QDO	41330.0	790.0	2*-SOH, 4*QDO
					40727.5	187.5	QDO
	conA	20456.5	186.0	QDO	40725.0	185.5	QDO
					40913.0	373.0	2*QDO
C49A	--	22929.5			45858.5		
		23187.0(*)					
	conB	23117.5	188.0	QDO	46234.0	375.5	2*QDO
		23100.0	170.5	QMO	46199.0	340.5	2*QMO
		23135.5	206.0	-SOH, QDO			
		23205.5(*)	18.5	-SOH			
		23376.5(*)	190.0	QDO			
	conA	ND	ND	ND	ND	ND	ND
C170A	--	22929.5			45858.5		
	conB	23117.5	187.0	QDO	46235.5	376.5	2*QDO
		23135.5	206.0	-SOH, QDO			
		23152.0	223.0	2*-SOH, QDO			
		23374.5[§]	445.0[§]	2*-SO_2H, 2*QDO[§]			
	conA	23151.5	222.5	2*-SOH, QDO	46303.0	444.5	4*-SOH, 2*QDO
		23194.5	265.0	conB			
C73A	--	22928.5			45856.0		
	conB	23287.5	359.0	QDO+QMO	45855.5	-0.5	--
		23305.0	376.5	2*QDO			
	conA	23115.5	187.0	QDO	46230.5	374.5	2*QDO
		23132.5	204.0	-SOH, QDO	46043.0	187.0	QDO
					46488.0	632.0	4*-SOH, 3*QDO
C49A/C73A/Δ171	--	20206.5			40413.5		
		20464.5(*)					
	conB	20394.5	188.0	QDO	40789.0	375.5	2*QDO
		20652.5(*)	188.0	QDO	41305.0[§]	891.5[§]	4*-SO_2H, 4*QDO[§]

		20414.0	207.5	-SOH, QDO			
	conA	20392.5	186.0	QDO	40785.0	371.5	2*QDO
		20411.0	204.5	-SOH, QDO	40820.5	407.0	2*-SOH, 2*QDO
		20429.5	223.0	$-SO_2H$, QDO	40860.0	446.5	4*-SOH, 2*QDO
		20650.5(*)	186.0	QDO	40742.0	328.5	Qx+QMO
		20669.0(*)	206.0	-SOH, QDO			
		20687.0(*)	223.0	2*-SOH, QDO			

QDO, quinoxaline 1,4-dioxide form of conoidin A

QMO, quinoxaline mono-oxide form of conoidin A

Qx, quinoxaline form of conoidin A

-SOH, oxidation to sulfenic acid

$-SO_2H$, oxidation to sulfinic acid

[§] suggests modification on a residue other than cysteine