Supplementary Materials

Table 1. *Chemical Shift Differences Between Oxidized and Reduced PRL-1*[a]

	Wild Type		*C170S-C171S*		Δδ (^{1}H)	Δδ (^{15}N)
	δ-^{1}H	δ-^{15}N	δ-^{1}H	δ-^{15}N		
M1					na	na
A2					na	na
R3					na	na
M4					na	na
N5					na	na
R6					na	na
P7					na	na
A8	8.45	126.0			na	na
P9					na	na
V10	8.52	118.9	8.05	118.2	0.47	0.7
E11	8.90	130.3	8.65	129.7	0.25	0.6
V12	9.29	126.9	9.21	126.9	0.08	0.0
T13	8.75	123.0	8.66	122.2	0.10	0.8
Y14	8.49	124.7	8.26	124.1	0.23	0.6
K15	9.00	125.6	8.85	125.4	0.15	0.2
N16	8.29	119.6	8.19	119.3	0.10	0.3
M17	8.52	119.4	8.32	119.3	0.19	0.1
R18	6.03	122.4	5.92	122.3	0.11	0.1
F19	9.36	121.0	9.19	121.1	0.18	-0.1
L20	8.94	124.0	8.56	123.7	0.38	0.3
I21	9.48	127.5	9.17	126.7	0.31	0.8
T22	8.01	120.8	7.81	120.3	0.19	0.5
H23	8.53	119.0	8.24	118.9	0.29	0.1
N24	8.66	120.3	8.00	118.1	0.66	2.2
P25					na	na
T26			6.85	104.9	na	na
N27					na	na
A28			8.28	126.1	na	na
T29			7.44	106.5	na	na
L30	7.51	124.6	7.30	124.5	0.21	0.1
N31	8.80	116.2	8.65	116.4	0.15	-0.2
K32	7.83	121.1	7.73	120.9	0.11	0.2
F33	8.15	122.8	7.88	122.5	0.27	0.3
I34	8.71	118.9	8.67	118.7	0.05	0.2
E35	7.79	118.4	7.64	118.5	0.15	-0.1
E36	7.98	121.0			na	na
L37	8.26	118.0	7.94	117.6	0.32	0.4
K38	8.24	122.2	8.35	117.9	-0.11	4.3
K39	7.98	122.2	7.80	121.9	0.19	0.3
Y40	7.54	114.6	7.35	114.7	0.19	-0.1
G41	8.07	109.7	7.90	109.0	0.17	0.7
V42	8.03	120.4	7.68	120.4	0.35	0.0
T43	7.95	112.4	7.94	113.0	0.02	-0.6
T44	7.53	120.3	7.45	120.5	0.08	-0.2
I45	9.45	127.1	9.29	125.9	0.16	1.2
V46	9.33	127.3	9.04	127.7	0.29	-0.4
R47	9.05	126.8	8.92	129.2	0.13	-2.4
V48	7.66	112.8	7.48	116.1	0.19	-3.3
C49	8.18	121.7	6.94	112.1	1.24	9.6
E50	8.57	127.4	8.94	119.2	-0.37	8.2
A51	8.56	127.4	8.27	126.1	0.29	1.3
T52	8.88	112.8			na	na
Y53	6.95	115.8	6.89	116.2	0.06	-0.4
D54	9.15	123.7	9.11	123.0	0.04	0.7
T55	8.78	115.6	8.40	116.4	0.38	-0.8
T56	8.70	120.9	8.54	119.4	0.16	1.5
L57	9.01	120.3	8.36	120.3	0.64	0.0
V58	7.15	117.1	7.02	117.4	0.13	-0.3
E59	8.58	119.2			na	na
K60	8.06	120.5	7.95	119.8	0.12	0.7
E61	7.49	116.6	7.35	116.5	0.14	0.1
G62	8.06	106.7	7.90	106.4	0.16	0.3
I63	7.24	121.6	6.99	121.4	0.25	0.2
H64	7.67	123.5	7.60	123.9	0.07	-0.4
V65	8.72	125.1	8.57	125.6	0.15	-0.5
L66	9.21	130.4	8.84	130.7	0.37	-0.3
D67	8.46	123.5	8.19	123.8	0.28	-0.3
W68	8.22	126.6	8.29	122.9	-0.07	3.7
P69					na	na
F70	8.41	119.9	7.28	113.9	1.13	6.0
D71	8.26	123.1	8.28	120.1	-0.02	3.0
D72	8.49	120.4	8.40	120.2	0.08	0.2
G73	8.69	108.6	7.57	111.2	1.12	-2.6
A74	7.72	123.9	7.89	123.4	-0.17	0.5
P75					na	na
P76					na	na
S77	7.77	116.8	8.34	121.6	-0.56	-4.8
N78	8.42	122.3	8.81	119.3	-0.40	3.0
Q79	8.21	118.6	8.09	118.6	0.12	0.0
I80	7.38	119.2	6.99	118.9	0.40	0.3
V81	7.86	119.3	7.65	118.5	0.20	0.8
D82	8.35	119.2	8.04	118.7	0.31	0.5
D83	8.54	122.4	8.16	122.2	0.38	0.2
W84	9.02	124.3	8.99	124.4	0.03	-0.1
L85	8.02		8.88	117.4	-0.86	na
S86	8.34	115.1	7.99	114.6	0.35	0.5
L87	8.07	123.7	7.89	123.3	0.18	0.4
V88	8.21	117.0	8.14	117.1	0.07	-0.1
K89	7.80	117.5	7.59	117.1	0.21	0.4
I90	8.03	116.6	7.74	116.2	0.29	0.4
K91	8.92	120.1	8.85	120.1	0.07	0.0
F92	7.24	111.5	7.07	111.2	0.17	0.3
R93	7.41	118.4	7.20	118.2	0.21	0.2
E94	8.64	117.5	8.41	117.3	0.22	0.2
E95	8.11	119.5	8.02	119.3	0.10	0.2
P96					na	na
G97	9.16	112.4	8.99	112.2	0.16	0.2
C98	8.24	117.6	8.05	117.4	0.19	0.2
C99	7.39	117.2	7.22	117.1	0.18	0.1
I100	8.39	128.3	8.31	128.1	0.08	0.2
A101	9.28	129.8	8.82	129.2	0.46	0.6
V102	9.06	118.5	9.11	120.5	-0.05	-2.0
H103	8.31	116.9	8.56	120.2	-0.24	-3.3
C104	8.54	121.9	8.33	122.9	0.21	-1.0

	Wild Type		*C170S-C171S*		Δδ (^{1}H)	Δδ (^{15}N)
	δ-^{1}H	δ-^{15}N	δ-^{1}H	δ-^{15}N		
V105			7.74	122.2	na	na
A106			8.28	126.1	na	na
G107	8.34	108.9	7.80	107.6	0.54	1.3
L108			8.18	120.6	na	na
G109			8.25	108.7	na	na
R110			8.25	108.8	na	na
A111					na	na
P112					na	na
V113	7.37	116.2	8.14	116.6	-0.77	-0.4
L114	7.54	116.1	7.11	117.9	0.43	-1.8
V115	6.90	118.0	7.45	119.2	-0.56	-1.2
A116	8.42	120.6	8.75	121.1	-0.33	-0.5
L117	8.15	115.6	8.02	115.8	0.14	-0.2
A118	7.73	119.2	7.63	119.3	0.10	-0.1
L119	7.90	117.2	7.89	117.2	0.01	0.0
I120	8.51	120.9	8.46	121.0	0.05	-0.1
E121	8.80	121.7	8.68	121.7	0.12	0.0
G122	8.23	106.0	8.12	105.9	0.11	0.1
G123	7.95	107.6	7.80	107.6	0.15	0.0
M124	8.60	123.5	8.54	123.9	0.06	-0.4
K125	8.87	123.8	8.73	123.9	0.14	-0.1
Y126	9.33	124.4	9.17	124.2	0.16	0.2
E127	9.39	118.8	9.20	119.1	0.19	-0.3
D128	6.95	118.5	6.76	118.3	0.18	0.2
A129	8.46	125.4	8.32	125.2	0.15	0.2
V130	8.03	114.9	8.02	115.9	0.01	-1.0
Q131	7.65	118.9	7.48	118.2	0.18	0.7
F132	8.59	120.5	8.49	120.6	0.10	-0.1
I133	7.74	115.8	7.88	116.0	-0.13	-0.2
R134	8.89	120.1	8.73	119.5	0.16	0.6
Q135	8.12	116.5	7.86	116.9	0.26	-0.4
K136	7.49	116.3	7.16	116.1	0.33	0.2
R137	8.08	120.7			na	na
R138					na	na
G139			8.86	108.8	na	na

	Wild Type		*C170S-C171S*		Δδ (^{1}H)	Δδ (^{15}N)
	δ-^{1}H	δ-^{15}N	δ-^{1}H	δ-^{15}N		
A140	7.62	121.0	7.30	121.4	0.32	-0.4
F141	8.12	110.8	8.16	110.9	-0.05	-0.1
N142	8.15	119.2			na	na
S143			8.24	115.9	na	na
K144					na	na
Q145			8.60	120.4	na	na
L146			8.43	119.4	na	na
L147	7.72	119.4	7.75	119.5	-0.03	-0.1
Y148	7.56	118.0	7.50	118.4	0.05	-0.4
L149	8.08	119.5	7.83	118.9	0.25	0.6
E150	8.15	120.2	8.04	120.6	0.11	-0.4
K151	7.15	114.4	7.13	114.2	0.01	0.2
Y152	7.29	123.0	7.10	123.4	0.19	-0.4
R153	7.78	130.2	7.56	130.2	0.22	0.0
P154					na	na
K155			9.90	122.9	na	na
M156			8.64	118.5	na	na
R157			8.54	118.1	na	na
L158			7.94	118.6	na	na
R159			7.52	120.3	na	na
F160			8.34	122.3	na	na
K161	7.49	116.4	8.26	122.7	-0.77	-6.3
D162	8.44	122.0	8.28	121.8	0.17	0.2
S163	8.27	115.8	8.11	115.7	0.16	0.1
N164	8.24	126.0	8.09	125.9	0.15	0.1
G165	8.47	114.5	8.32	114.3	0.15	0.2
H166	7.83	125.7	8.16	119.2	-0.33	6.5
R167					na	na
N168					na	na
N169					na	na
S170			8.27	116.4	na	na
S171			8.26	117.7	na	na
I172			7.99	122.2	na	na
Q173			7.86	128.9	na	na
			average ($\Delta\delta_{avg}$)		0.24	0.9

[a]Δδ were calculated by subtracting the chemical shift of PRL-1-C170S-C171S from those for PRL-1-WT. $\Delta\delta_{avg}$ was calculated by averaging the absolute values of each Δδ. All chemical shift information was taken from BMRB entries 6080 and 15949 for PRL-1-WT and PRL-1-C170S-C171S, respectively.

Figure Legends

Figure 1. 2 mg/mL PRL-1-WT was incubated with increasing concentrations of reduced glutathione (GSH) and run on SDS-PAGE as describe above. Although, only 10-50 mM GSH is shown here, concentrations spanning 0.1-200 mM GSH were tested. The migration pattern of PRL-1-WT in the presence of greater than 50 mM GSH was identical to that of the 50 mM data point. All samples for both panels contained 50 mM sodium phosphate buffer and 100 mM NaCl at pH 7.5.

Figure 2. *Mass spectra of PRL-1 fragments from a tryptic digest after LC/MS separation.* The column on the left contains data corresponding to the mass series for the C49-containing peptide and the right shows the disulfide bound C49-C104 containing peptides. WT PRL-1 is shown in row **A** and PRL-1-C170S-C171S in row **B**. Some spectra are expanded vertically and the expansion factor is indicated with an arrow in each panel.

Figure 3. The spectrum of the oxidized form of PRL-1-C98A (red) is overlaid with reduced PRL-1-C98A (black). Reduction was accomplished by the addition of 10 mM DTT. A significant conformational change is observed in PRL-1 upon reduction, where greater than 90% of the peaks move. The C49 resonance moves significantly up-field upon addition of DTT and can be used as a diagnostic to assess whether the protein is oxidized or reduced. The C49 residue in both oxidized (red) and reduced (black) spectra are highlighted in boxes.

Figures

Figure 1

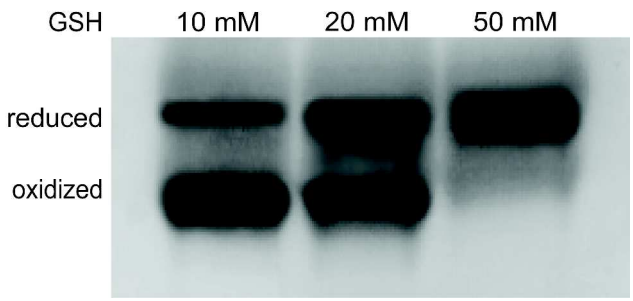


Figure 2

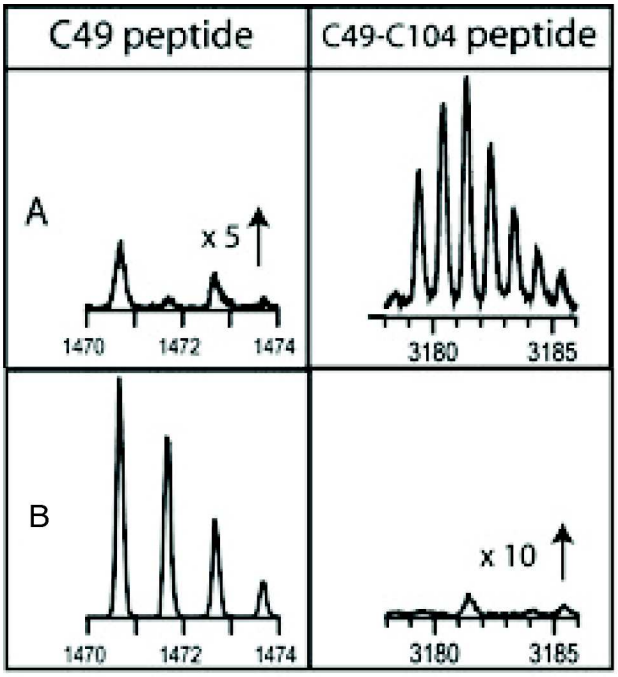
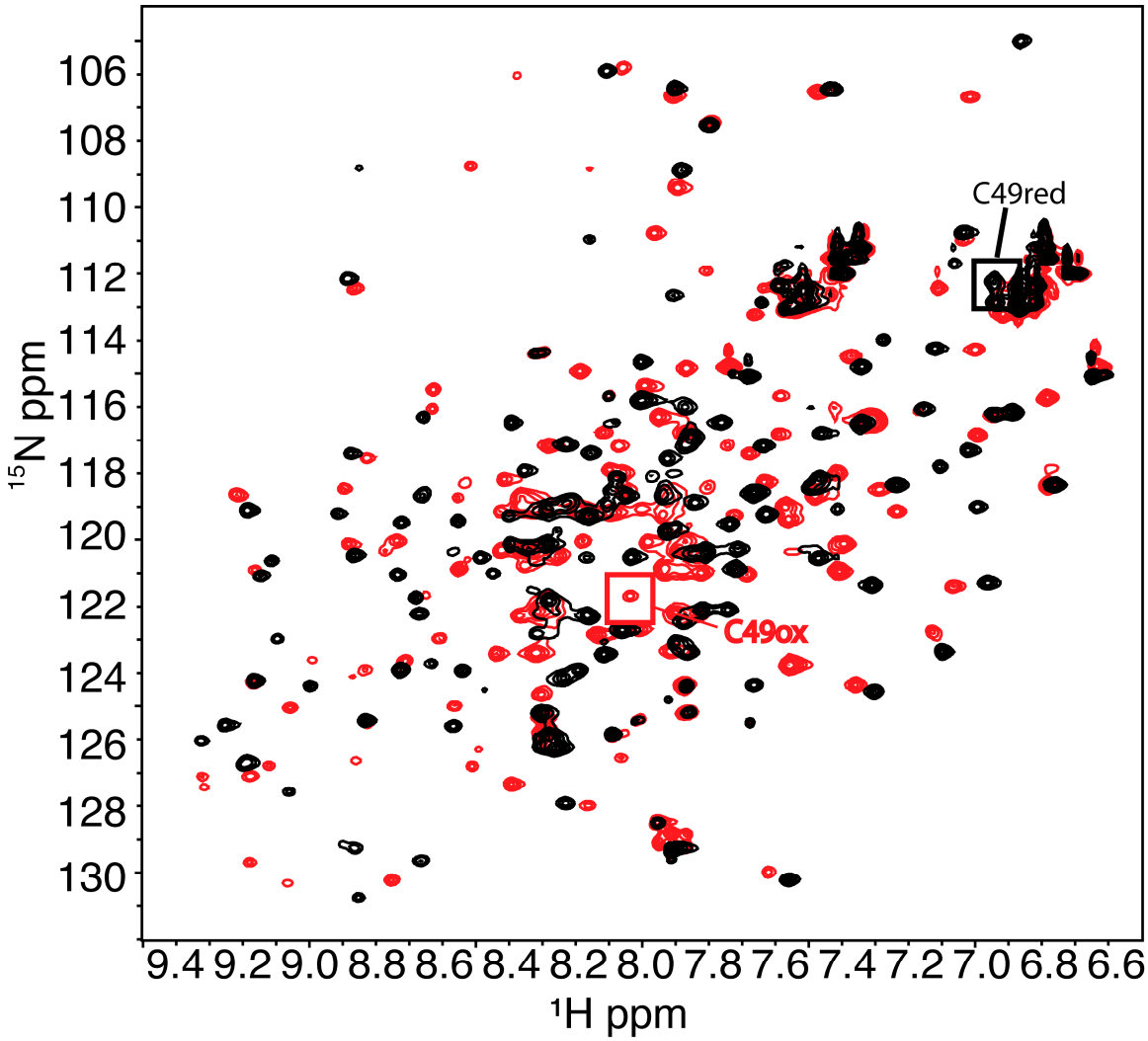


Figure 3


C49red
C49ox
106
108
110
112
114
116
118
120
122
124
126
128
130
15N ppm
9.4 9.2 9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6
1H ppm