

Are Granulins Copper Sequestering Proteins?

Supplementary information

Fig S1

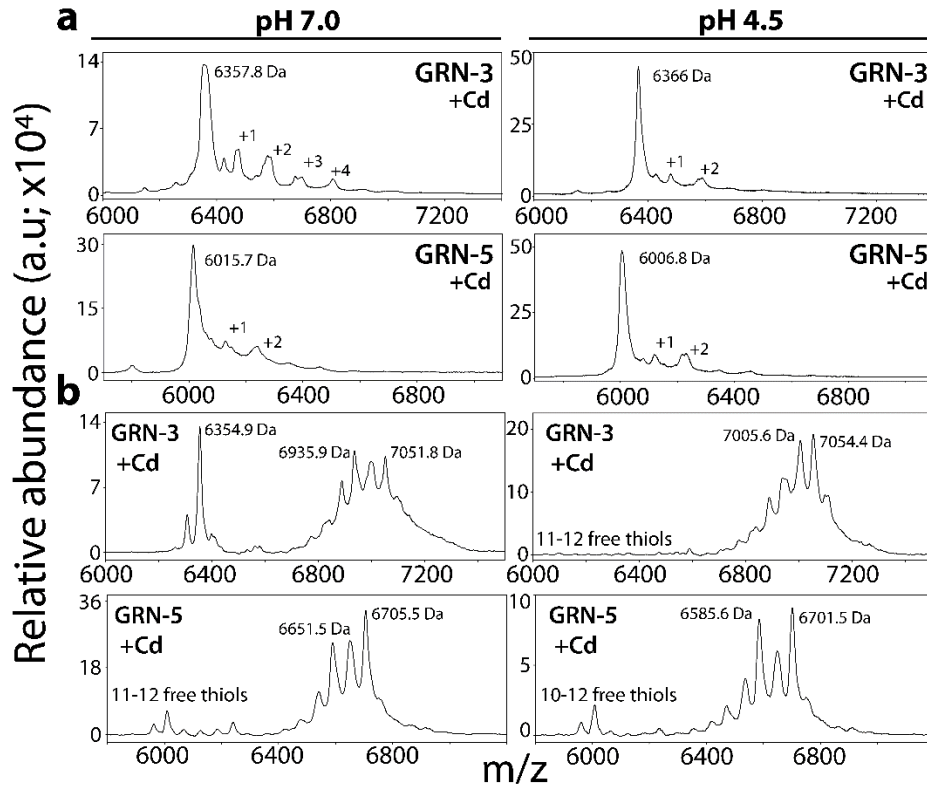


Fig S1: Determination of Cd binding to GRNs and its effects on redox state of thiols. a) MALDI-MS spectra of GRN-3,5 incubated separately with Cd at neutral or acidic pH. b) Alkylation reactions were carried out by incubating samples from a) with 1 mM IAA and analyzed using MALDI-MS.

GRN-3 Ctrl -pH 7**Cu**

	Theoretical (Da)	Observed		Theoretical (Da)
0-apo	6367.3	6367.8	0-apo	6367.3
			1 (plus 63.5 Da)	6430.8
			2	6494.3
			3	6557.8
			4	6621.3
			5	6684.8
			6	6748.3
			7	6811.8
			8	6875.3
			9	6938.8
			10	7002.3
			11	7065.8
			12	7129.3

GRN-5 Ctrl -pH 7**Cu**

	Theoretical (Da)	Observed		Theoretical (Da)
0	6017.7	6017.8	0-apo	6017.7
			1 (plus 63.5 Da)	6081.2
			2	6144.7
			3	6208.2
			4	6271.7
			5	6335.2
			6	6398.7
			7	6462.2
			8	6525.7
			9	6589.2
			10	6652.7
			11	6716.2
			12	6779.7

Zn

Observed	Difference		Theoretical (Da)
6357.8		9.5	0-apo 6367.3
6421.1		9.7	1 (plus 65.3 Da) 6432.6
6485.3		9	2 6497.9
6549		8.8	3 6563.2
6612.1		9.2	4 6628.5
6675.4		9.4	5 6693.8
6738.7		9.6	
6802		9.8	
6865.8		9.5	
6929.3		9.5	
6993.1		9.2	
7056.3		9.5	
7119.9		9.4	

Observed	Difference	
6001.4		16.3
6064.3		16.9
6130.5		14.2
6208.8		-0.6
6256.4		15.3
6320.5		14.7
6384.1		14.6
6447.6		14.6
6511.1		14.6
6574.5		14.7
6638.7		14
6702.2		14
6766.1		13.6

Cd

Observed	Difference		Theoretical (Da)
6370.9		-3.6 0-apo	6367.3
6435.6		-3 1 (plus 112.4 Da)	6479.7
6501		-3.1	2 6592.1
6566.3		-3.1	3 6704.5
6626.8		1.7	4 6816.9
6686.1		7.7	

Cd

	Theoretical (Da)
0-apo	6017.7
1 (plus 112.4 Da)	6130.1
2	6242.5
3	6354.9

Observed	Difference
6357.8	9.5
6474.1	5.6
6577.9	14.2
6691.3	13.2
6803.5	13.4

Observed	Difference
6015.7	2
6128.5	1.6
6236.5	6
6347.5	7.4

GRN-3 Ctrl -pH 4.5**Cu**

	Theoretical (Da)	Observed		Theoretical (Da)	Observed
0-apo	6367.3	6365.2	0-apo	6367.3	6352.7
			1 (plus 63.5 Da)	6430.8	6417.2
			2	6494.3	6480.2
			3	6557.8	6560.6
			4	6621.3	6608.8
			5	6684.8	6670.7
			6	6748.3	6733.3
			7	6811.8	6799.3
			8	6875.3	
			9	6938.8	
			10	7002.3	
			11	7065.8	
			12	7129.3	

GRN-5 Ctrl -pH 4.5**Cu**

	Theoretical (Da)	Observed		Theoretical (Da)	Observed
0	6017.7	6016.2	0-apo	6017.7	6004.8
			1 (plus 63.5 Da)	6081.2	6069.1
			2	6144.7	
			3	6208.2	
			4	6271.7	
			5	6335.2	
			6	6398.7	
			7	6462.2	
			8	6525.7	
			9	6589.2	
			10	6652.7	
			11	6716.2	
			12	6779.7	

Cd

Difference		Theoretical (Da)	Observed	Difference
14.6	0-apo	6367.3	6366	1.3
13.6	1 (plus 112.4 Da)	6479.7	6478.7	1
14.1		6592.1	6588.3	3.8
-2.8				
12.5				
14.1				
15				
12.5				

Cd

Difference		Theoretical (Da)	Observed	Difference
12.9	0-apo	6017.7	6006.8	10.9
12.1	1 (plus 112.4 Da)	6130.1	6120.7	9.4
		6242.5	6230.7	11.8

GRN-3 IAS pH 7**Ctrl**

Theoretical Observed

No free thiols	6367.3	
1 free thiol (plus 58 Da)	6425.3	
2 free thiol	6483.3	
3 free thiol	6541.3	
4 free thiol	6599.3	
5 free thiol	6657.3	
6 free thiol	6715.3	
7 free thiol	6773.3	
8 free thiol	6831.3	6843.8
9 free thiol	6889.3	6891.9
10 free thiol	6947.3	6957.9
11 free thiol	7005.3	7007.9
12 free thiol	7063.3	7055.7
		7009.1

Ca

Theoretical

No free thiols	6367.3
1 free thiol (plus 58 Da)	6425.3
2 free thiol	6483.3
3 free thiol	6541.3
4 free thiol	6599.3
5 free thiol	6657.3
6 free thiol	6715.3
7 free thiol	6773.3
-12.5 8 free thiol	6831.3
-2.6 9 free thiol	6889.3
-10.6 10 free thiol	6947.3
-2.6 11 free thiol	7005.3
7.6 12 free thiol	7063.3

GRN-5 IAS pH 7**Ctrl**

Theoretical Observed Difference

No free thiols	6017.7	6014.6	5968.1
1 free thiol (plus 58 Da)	6075.7	6070.3	
2 free thiol	6133.7	6131.5	
3 free thiol	6191.7	6194.5	
4 free thiol	6249.7	6248	
5 free thiol	6307.7	6315	
6 free thiol	6365.7	6370.4	
7 free thiol	6423.7	6428.6	
8 free thiol	6481.7	6482.3	
9 free thiol	6539.7	6540.9	
10 free thiol	6597.7	6592.9	
11 free thiol	6655.7	6646.7	
12 free thiol	6713.7	6704.6	

Ca

Theoretical

No free thiols	6017.7
5.4 1 free thiol (plus 58 Da)	6075.7
2.2 2 free thiol	6133.7
-2.8 3 free thiol	6191.7
1.7 4 free thiol	6249.7
-7.3 5 free thiol	6307.7
-4.7 6 free thiol	6365.7
-4.9 7 free thiol	6423.7
-0.6 8 free thiol	6481.7
-1.2 9 free thiol	6539.7
4.8 10 free thiol	6597.7
9 11 free thiol	6655.7
9.1 12 free thiol	6713.7

Observed	Difference	Co	Theoretical	Observed	Difference	Cu
		No free thiols	6367.3			No free thiols
		1 free thiol (plus 58 Da)	6425.3			1 free thiol (plus 58 Da)
		2 free thiol	6483.3			2 free thiol
		3 free thiol	6541.3			3 free thiol
		4 free thiol	6599.3			4 free thiol
		5 free thiol	6657.3			5 free thiol
		6 free thiol	6715.3			6 free thiol
		7 free thiol	6773.3	6785	-11.7	7 free thiol
6842.5	-11.2	8 free thiol	6831.3	6846.7	-15.4	8 free thiol
6890.6	-1.3	9 free thiol	6889.3	6898.4	-9.1	9 free thiol
6956.6	-9.3	10 free thiol	6947.3	6946.9	0.4	10 free thiol
7006.9	-1.6	11 free thiol	7005.3	7014.9	-9.6	11 free thiol
7055.5	7.8	12 free thiol	7063.3	7064.5	-1.2	12 free thiol
7110.9				7112.9		

Observed	Difference	Co	Theoretical	Observed	Difference	Cu
5962.5				5961.6		
6009.5	8.2	No free thiols	6017.7	6008.4	9.3	No free thiols
6066	9.7	1 free thiol (plus 58 Da)	6075.7	6064.4	11.3	1 free thiol (plus 58 Da)
6125.8	7.9	2 free thiol	6133.7	6123.3	10.4	2 free thiol
6183.9	7.8	3 free thiol	6191.7	6189.7	2	3 free thiol
6243.5	6.2	4 free thiol	6249.7	6243.7	6	4 free thiol
6311.3	-3.6	5 free thiol	6307.7	6307.2	0.5	5 free thiol
6370.2	-4.5	6 free thiol	6365.7	6366.1	-0.4	6 free thiol
6428.4	-4.7	7 free thiol	6423.7	6423.3	0.4	7 free thiol
6477.1	4.6	8 free thiol	6481.7	6471.1	10.6	8 free thiol
6534.3	5.4	9 free thiol	6539.7	6541.2	-1.5	9 free thiol
6591.85	5.85	10 free thiol	6597.7	6589.2	8.5	10 free thiol
6647.2	8.5	11 free thiol	6655.7	6646.8	8.9	11 free thiol
6704.3	9.4	12 free thiol	6713.7	6706	7.7	12 free thiol
				7112.9		

Theoretical	Observed	Difference	Mg	Theoretical	Observed	Difference
6367.3	6380.7	-13.4	No free thiols	6367.3		
6425.3	6428.4	-3.1	1 free thiol (plus 58 Da)	6425.3		
6483.3	6490.8	-7.5	2 free thiol	6483.3		
6541.3	6543.7	-2.4	3 free thiol	6541.3		
6599.3	6589.4	9.9	4 free thiol	6599.3		
6657.3			5 free thiol	6657.3		
6715.3			6 free thiol	6715.3		
6773.3			7 free thiol	6773.3		
6831.3			8 free thiol	6831.3	6843.3	-12
6889.3			9 free thiol	6889.3	6891.4	-2.1
6947.3			10 free thiol	6947.3	6956.9	-9.6
7005.3			11 free thiol	7005.3	7005.8	-0.5
7063.3			12 free thiol	7063.3	7054.3	9
					7113.39	

Theoretical	Observed	Difference	Mg	Theoretical	Observed	Difference
	5961.4				5959.8	
6017.7	6009	8.7	No free thiols	6017.7	6007.1	10.6
6075.7	6058.8	16.9	1 free thiol (plus 58 Da)	6075.7	6065.7	10
6133.7	6125.9	7.8	2 free thiol	6133.7	6124.3	9.4
6191.7			3 free thiol	6191.7	6187.2	4.5
6249.7			4 free thiol	6249.7	6238.7	11
6307.7			5 free thiol	6307.7	6307.9	-0.2
6365.7			6 free thiol	6365.7	6366.6	-0.9
6423.7			7 free thiol	6423.7	6426.2	-2.5
6481.7			8 free thiol	6481.7	6484.4	-2.7
6539.7			9 free thiol	6539.7	6542.3	-2.6
6597.7			10 free thiol	6597.7	6589.2	8.5
6655.7			11 free thiol	6655.7	6648.9	6.8
6713.7			12 free thiol	6713.7	6707.4	6.3

Mn				Ni			
	Theoretical	Observed	Difference		Theoretical	Observed	Difference
No free thiols	6367.3			No free thic	6367.3		
1 free thiol (plus 58 Da)	6425.3			1 free thiol	6425.3		
2 free thiol	6483.3			2 free thiol	6483.3		
3 free thiol	6541.3			3 free thiol	6541.3		
4 free thiol	6599.3	6609	-9.7	4 free thiol	6599.3		
5 free thiol	6657.3	6672.8	-15.5	5 free thiol	6657.3		
6 free thiol	6715.3	6721.6	-6.3	6 free thiol	6715.3		
7 free thiol	6773.3	6774.3	-1	7 free thiol	6773.3		
8 free thiol	6831.3	6836	-4.7	8 free thiol	6831.3	6843.5	-12.2
9 free thiol	6889.3	6889.8	-0.5	9 free thiol	6889.3	6892.1	-2.8
10 free thiol	6947.3	6939	8.3	10 free thic	6947.3	6959.2	-11.9
11 free thiol	7005.3			11 free thic	7005.3	7011.2	-5.9
12 free thiol	7063.3			12 free thic	7063.3	7057.8	5.5
						7113.89	

Mn				Ni			
	Theoretical	Observed	Difference		Theoretical	Observed	Difference
		5962.3				5963.6	
No free thiols	6017.7	6010.2	7.5	No free thic	6017.7	6010	7.7
1 free thiol (plus 58 Da)	6075.7	6066.2	9.5	1 free thiol	6075.7	6065.6	10.1
2 free thiol	6133.7	6125.8	7.9	2 free thiol	6133.7	6126.9	6.8
3 free thiol	6191.7	6187.9	3.8	3 free thiol	6191.7	6196.4	-4.7
4 free thiol	6249.7	6241.5	8.2	4 free thiol	6249.7	6255.7	-6
5 free thiol	6307.7	6308.8	-1.1	5 free thiol	6307.7	6312.7	-5
6 free thiol	6365.7	6366.8	-1.1	6 free thiol	6365.7	6373.3	-7.6
7 free thiol	6423.7	6424.7	-1	7 free thiol	6423.7	6429.2	-5.5
8 free thiol	6481.7	6483.4	-1.7	8 free thiol	6481.7	6476.4	5.3
9 free thiol	6539.7	6541.1	-1.4	9 free thiol	6539.7	6532.5	7.2
10 free thiol	6597.7	6590.1	7.6	10 free thic	6597.7	6592.5	5.2
11 free thiol	6655.7	6648.1	7.6	11 free thic	6655.7	6638.8	16.9
12 free thiol	6713.7	6706.9	6.8	12 free thic	6713.7	6702.7	11

Zn			Cd				
Theoretical	Observed	Difference	Theoretical	Observed	Difference		
No free thic	6367.3		No free thic	6367.3	6354.9	12.4	
1 free thiol	6425.3		1 free thiol	6425.3			
2 free thiol	6483.3	6474.1	9.2	2 free thiol	6483.3		
3 free thiol	6541.3	6521.6	19.7	3 free thiol	6541.3		
4 free thiol	6599.3	6569.9	29.4	4 free thiol	6599.3		
5 free thiol	6657.3	6688.5	-31.2	5 free thiol	6657.3		
6 free thiol	6715.3	6738.1	-22.8	6 free thiol	6715.3		
7 free thiol	6773.3	6785.3	-12	7 free thiol	6773.3		
8 free thiol	6831.3	6840.3	-9	8 free thiol	6831.3	6839.1	-7.8
9 free thiol	6889.3	6896.3	-7	9 free thiol	6889.3	6888.3	1
10 free thic	6947.3	6964.7	-17.4	10 free thic	6947.3	6935.9	11.4
11 free thic	7005.3	7013.7	-8.4	11 free thic	7005.3	6999.1	6.2
12 free thic	7063.3	7062.5	0.8	12 free thic	7063.3	7051.8	11.5
		7111.1				7094.2	

Zn			Cd				
Theoretical	Observed	Difference	Theoretical	Observed	Difference		
	5961.6			5962.6			
No free thic	6017.7	6009.8	7.9	No free thic	6017.7	6009.4	8.3
1 free thiol	6075.7	6067.3	8.4	1 free thiol	6075.7		
2 free thiol	6133.7	6126.4	7.3	2 free thiol	6133.7		
3 free thiol	6191.7	6188.2	3.5	3 free thiol	6191.7		
4 free thiol	6249.7	6240.9	8.8	4 free thiol	6249.7	6240.9	8.8
5 free thiol	6307.7		6307.7	5 free thiol	6307.7		
6 free thiol	6365.7	6375.3	-9.6	6 free thiol	6365.7		
7 free thiol	6423.7	6431.8	-8.1	7 free thiol	6423.7		
8 free thiol	6481.7	6490.4	-8.7	8 free thiol	6481.7	6479	2.7
9 free thiol	6539.7	6543.5	-3.8	9 free thiol	6539.7	6541.1	-1.4
10 free thic	6597.7	6601.3	-3.6	10 free thic	6597.7	6589.9	7.8
11 free thic	6655.7	6660.1	-4.4	11 free thic	6655.7	6651.5	4.2
12 free thic	6713.7	6709.3	4.4	12 free thic	6713.7	6705.5	8.2
						6750.2	

GRN-3 IAS pH 4.5

Ctrl				Ca				Co
	Theoretical	Observed	Difference		Theoretical	Observed	Difference	
No free thic	6367.3			No free thic	6367.3			No free thic
1 free thiol	6425.3			1 free thiol	6425.3			1 free thiol
2 free thiol	6483.3	6496.1	-12.8	2 free thiol	6483.3	6499.4	-16.1	2 free thiol
3 free thiol	6541.3	6547.7	-6.4	3 free thiol	6541.3	6551.5	-10.2	3 free thiol
4 free thiol	6599.3	6601.6	-2.3	4 free thiol	6599.3	6604	-4.7	4 free thiol
5 free thiol	6657.3	6657.5	-0.2	5 free thiol	6657.3	6659.7	-2.4	5 free thiol
6 free thiol	6715.3	6714.9	0.4	6 free thiol	6715.3	6716.6	-1.3	6 free thiol
7 free thiol	6773.3	6772.9	0.4	7 free thiol	6773.3	6774.1	-0.8	7 free thiol
8 free thiol	6831.3	6830.1	1.2	8 free thiol	6831.3	6831.4	-0.1	8 free thiol
9 free thiol	6889.3	6886.1	3.2	9 free thiol	6889.3	6888.2	1.1	9 free thiol
10 free thic	6947.3	6923.5	23.8	10 free thic	6947.3	6941.9	5.4	10 free thic
11 free thic	7005.3			11 free thic	7005.3			11 free thic
12 free thic	7063.3			12 free thic	7063.3			12 free thic

GRN-5 IAS pH 4.5

Ctrl				Ca				Co
	Theoretical	Observed	Difference		Theoretical	Observed	Difference	
		5965.4				5964		
No free thic	6017.7	6012.8	4.9	No free thic	6017.7	6012.8	4.9	No free thic
1 free thiol	6075.7	6076	-0.3	1 free thiol	6075.7	6076.6	-0.9	1 free thiol
2 free thiol	6133.7	6135.4	-1.7	2 free thiol	6133.7	6149.7	-16	2 free thiol
3 free thiol	6191.7	6192.7	-1	3 free thiol	6191.7	6195.1	-3.4	3 free thiol
4 free thiol	6249.7	6250.7	-1	4 free thiol	6249.7	6252.6	-2.9	4 free thiol
5 free thiol	6307.7	6308.6	-0.9	5 free thiol	6307.7	6310.5	-2.8	5 free thiol
6 free thiol	6365.7	6364.9	0.8	6 free thiol	6365.7	6368.1	-2.4	6 free thiol
7 free thiol	6423.7			7 free thiol	6423.7	6423.8	-0.1	7 free thiol
8 free thiol	6481.7			8 free thiol	6481.7			8 free thiol
9 free thiol	6539.7			9 free thiol	6539.7			9 free thiol
10 free thic	6597.7			10 free thic	6597.7			10 free thic
11 free thic	6655.7			11 free thic	6655.7			11 free thic
12 free thic	6713.7			12 free thic	6713.7			12 free thic

Theoretical	Observed	Difference	Cu	Theoretical	Observed	Difference	Mg	Theoretical
6367.3			No free thic	6367.3	6364.4	2.9	No free thic	6367.3
6425.3			1 free thiol	6425.3	6426.1	-0.8	1 free thiol	6425.3
6483.3	6498.5	-15.2	2 free thiol	6483.3			2 free thiol	6483.3
6541.3	6553.5	-12.2	3 free thiol	6541.3			3 free thiol	6541.3
6599.3	6609	-9.7	4 free thiol	6599.3			4 free thiol	6599.3
6657.3	6662	-4.7	5 free thiol	6657.3			5 free thiol	6657.3
6715.3	6717.5	-2.2	6 free thiol	6715.3			6 free thiol	6715.3
6773.3	6773	0.3	7 free thiol	6773.3			7 free thiol	6773.3
6831.3	6830	1.3	8 free thiol	6831.3			8 free thiol	6831.3
6889.3	6886.8	2.5	9 free thiol	6889.3			9 free thiol	6889.3
6947.3	6942.9	4.4	10 free thic	6947.3			10 free thic	6947.3
7005.3	6995.8	9.5	11 free thic	7005.3			11 free thic	7005.3
7063.3	7050.9	12.4	12 free thic	7063.3			12 free thic	7063.3

Theoretical	Observed	Difference	Cu	Theoretical	Observed	Difference	Mg	Theoretical
	5966.8				5965.8			
6017.7	6014.1	3.6	No free thic	6017.7	6014.9	2.8	No free thic	6017.7
6075.7	6073.8	1.9	1 free thiol	6075.7	6061	14.7	1 free thiol	6075.7
6133.7	6146	-12.3	2 free thiol	6133.7	6133.5	0.2	2 free thiol	6133.7
6191.7	6192.3	-0.6	3 free thiol	6191.7			3 free thiol	6191.7
6249.7	6249.3	0.4	4 free thiol	6249.7			4 free thiol	6249.7
6307.7	6306.2	1.5	5 free thiol	6307.7			5 free thiol	6307.7
6365.7	6363.6	2.1	6 free thiol	6365.7			6 free thiol	6365.7
6423.7	6418.9	4.8	7 free thiol	6423.7			7 free thiol	6423.7
6481.7			8 free thiol	6481.7			8 free thiol	6481.7
6539.7			9 free thiol	6539.7			9 free thiol	6539.7
6597.7			10 free thic	6597.7			10 free thic	6597.7
6655.7			11 free thic	6655.7			11 free thic	6655.7
6713.7			12 free thic	6713.7			12 free thic	6713.7

Observed	Difference	Mn	Theoretical	Observed	Difference	Ni	Theoretical	Observed
		No free thic	6367.3			No free thic	6367.3	
		1 free thiol	6425.3			1 free thiol	6425.3	
6495.7	-12.4	2 free thiol	6483.3	6494	-10.7	2 free thiol	6483.3	6489.9
6552.1	-10.8	3 free thiol	6541.3	6549.5	-8.2	3 free thiol	6541.3	6548.5
6606.8	-7.5	4 free thiol	6599.3	6605.1	-5.8	4 free thiol	6599.3	6603.3
6658.9	-1.6	5 free thiol	6657.3	6658.3	-1	5 free thiol	6657.3	6658
6713.5	1.8	6 free thiol	6715.3	6713.5	1.8	6 free thiol	6715.3	6712.7
6770.1	3.2	7 free thiol	6773.3	6769.3	4	7 free thiol	6773.3	6767.9
6827.7	3.6	8 free thiol	6831.3	6826.5	4.8	8 free thiol	6831.3	6825.5
6885.4	3.9	9 free thiol	6889.3	6884.3	5	9 free thiol	6889.3	6884.1
6942.6	4.7	10 free thic	6947.3	6941.6	5.7	10 free thic	6947.3	6941.3
6998.3	7	11 free thic	7005.3	6996.4	8.9	11 free thic	7005.3	6987.5
7036.7	26.6	12 free thic	7063.3	7034.8	28.5	12 free thic	7063.3	7035

Observed	Difference	Mn	Theoretical	Observed	Difference	Ni	Theoretical	Observed
5967.6				5964.4				5966.2
6016.2	1.5	No free thic	6017.7	6012.7	5	No free thic	6017.7	6013.8
6077.2	-1.5	1 free thiol	6075.7	6072.6	3.1	1 free thiol	6075.7	6073.5
6150.7	-17	2 free thiol	6133.7	6134.8	-1.1	2 free thiol	6133.7	6133.2
6197.8	-6.1	3 free thiol	6191.7	6192.7	-1	3 free thiol	6191.7	6191.7
6254.4	-4.7	4 free thiol	6249.7	6250.1	-0.4	4 free thiol	6249.7	6249.1
6311.4	-3.7	5 free thiol	6307.7	6307.5	0.2	5 free thiol	6307.7	6307
6368.9	-3.2	6 free thiol	6365.7	6365.3	0.4	6 free thiol	6365.7	6364.7
6425.3	-1.6	7 free thiol	6423.7	6422.5	1.2	7 free thiol	6423.7	6423
		8 free thiol	6481.7			8 free thiol	6481.7	
		9 free thiol	6539.7			9 free thiol	6539.7	
		10 free thic	6597.7			10 free thic	6597.7	
		11 free thic	6655.7			11 free thic	6655.7	
		12 free thic	6713.7			12 free thic	6713.7	

