Supplementary information

Modulation of Protein-Graphene Oxide Interactions with varying degrees of Oxidation.

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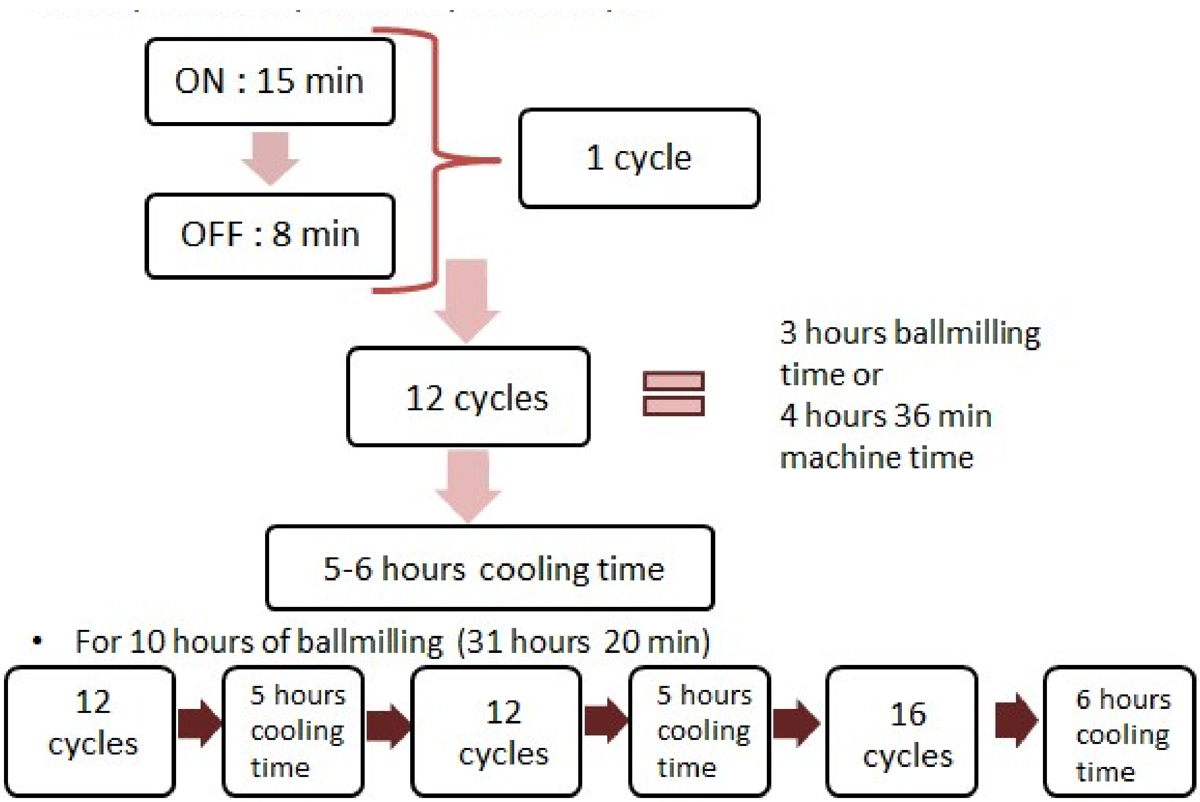
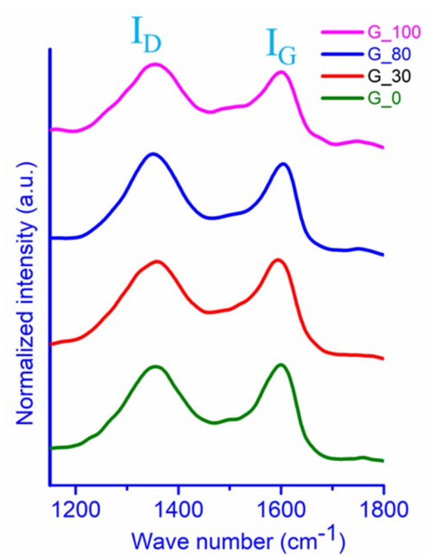
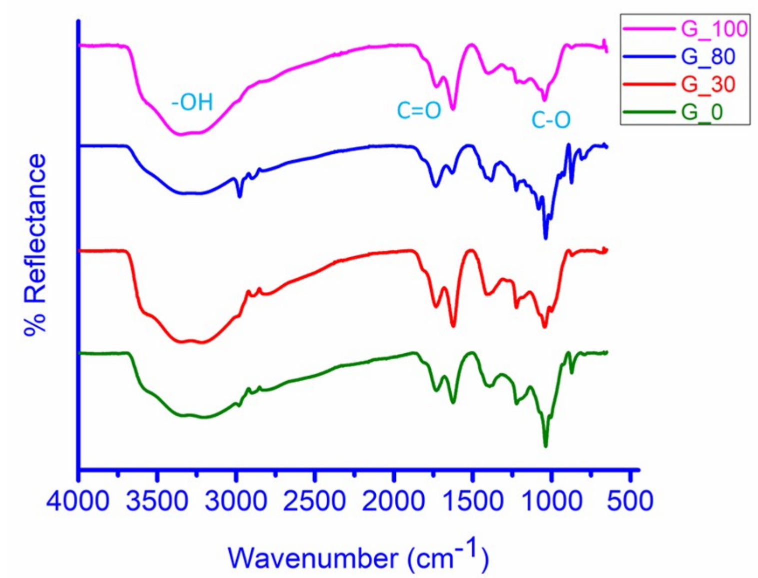


Figure S1: Schematic representation of high energy planetary ball milling process to introduce defects in graphite.

Characterization of GO with varying amounts of defects

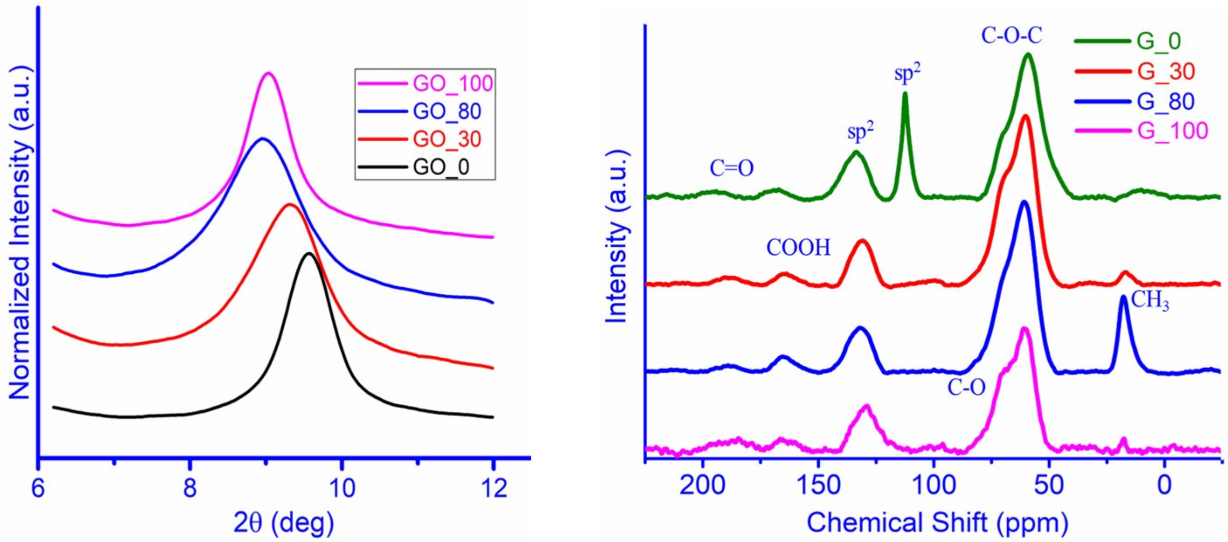


I_D
I_G
G_100
G_80
G_30
G_0
Normalized intensity (a.u.)
1200
1400
1600
1800
Wave number (cm-1)



G_100
G_80
G_30
G_0
-OH
C=O
C-O
% Reflectance
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)

Figure S2: Raman spectra (left) and FTIR spectra (right) of different GO samples in different colors**.**



GO_100
GO_80
GO_30
GO_0
Normalized Intensity (a.u.)
2θ (deg)
6
8
10
12
G_0
G_30
G_80
G_100
C-O-C
sp²
sp²
C=O
COOH
CH₃
C-O
Intensity (a.u.)
Chemical Shift (ppm)
200
150
100
50
0

Figure S3: XRD (left) and ss-NMR spectra (right) of different GO samples shown in different colors.

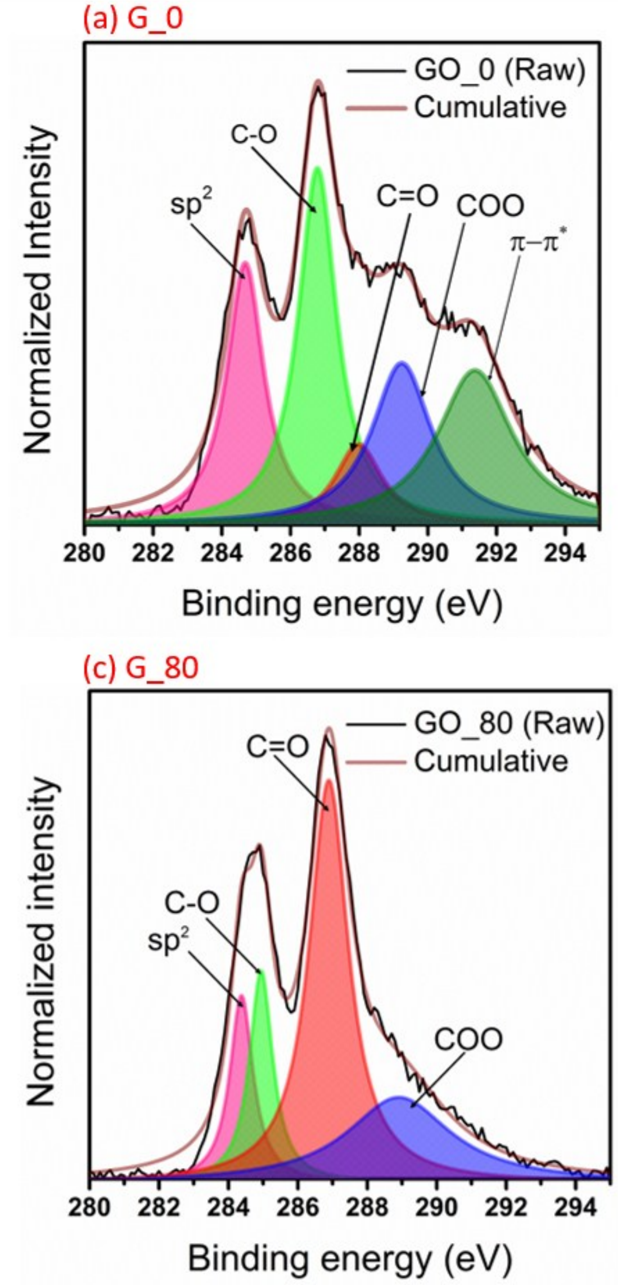


Figure S4: The Deconvolution of XPS High Resolution C1s Spectra of G_0 (a), G_30 (b), G_80 (c) and G_100 (d).

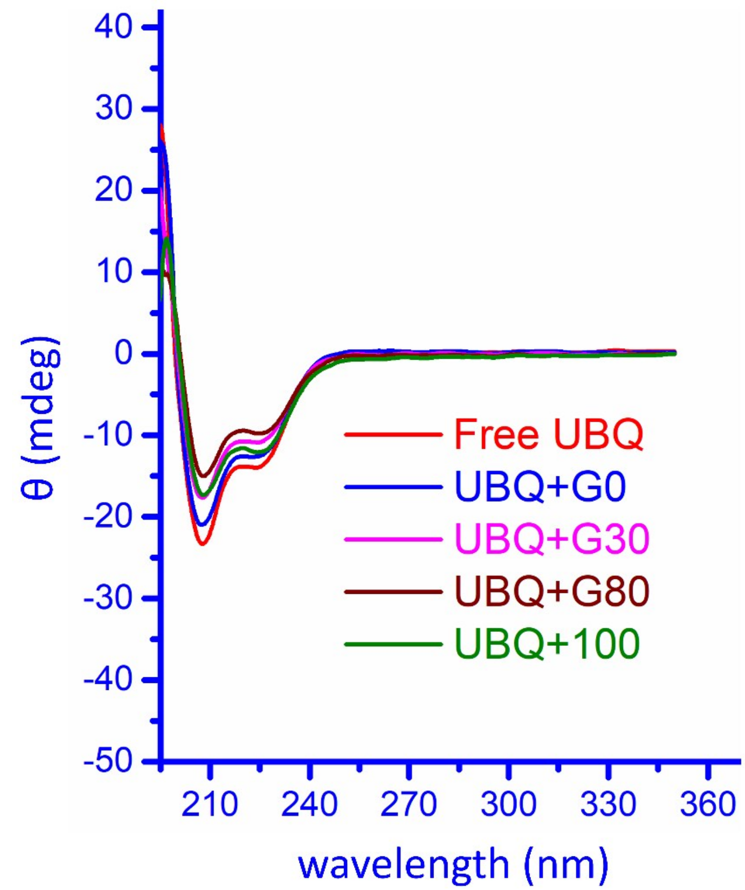
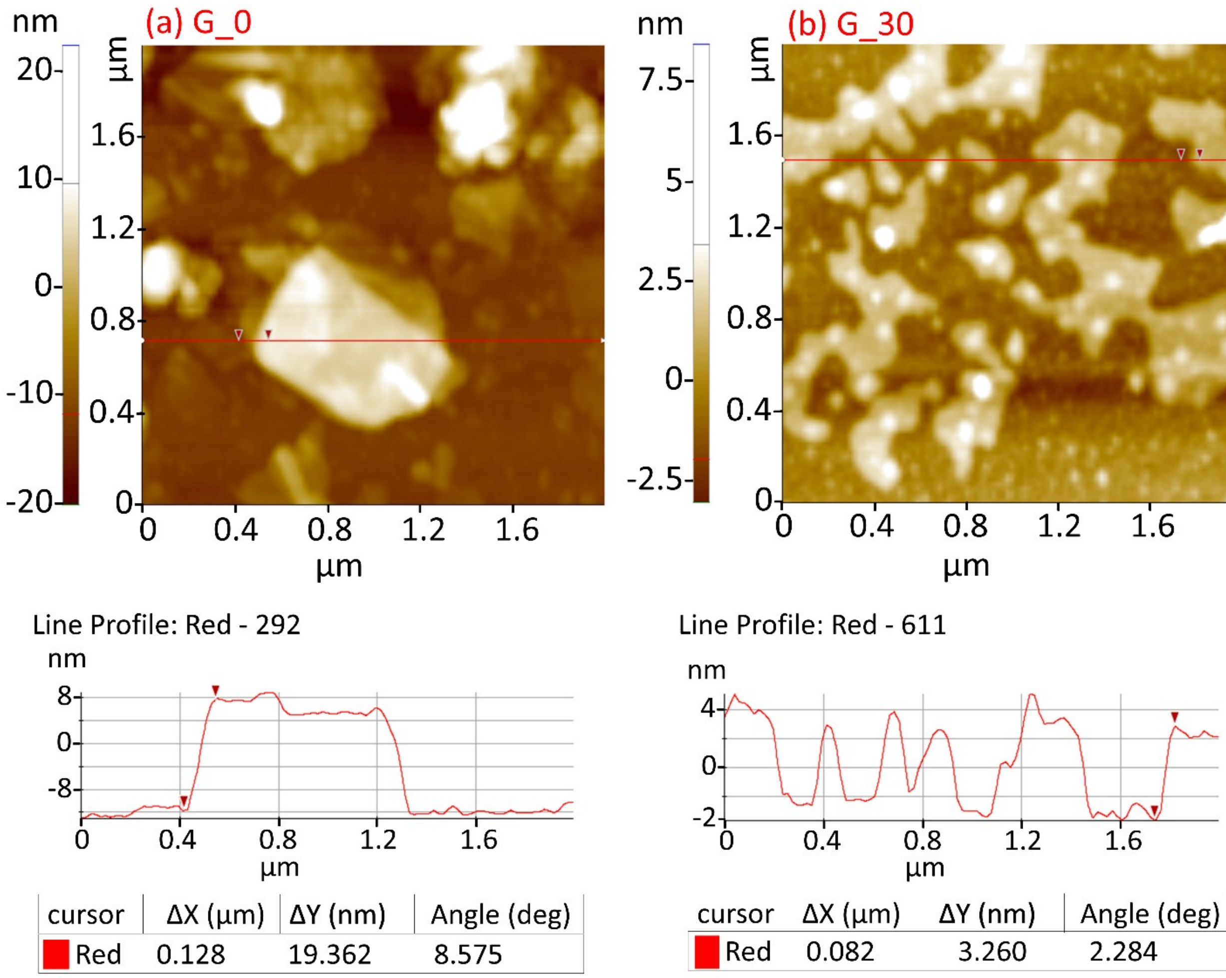


Figure S5: CD spectra of free ubiquitin and in conjugation with different GO samples.


(a) G_0
(b) G_30
nm
µm
Line Profile: Red - 292
Line Profile: Red - 611
cursor ΔX (µm) ΔY (nm) Angle (deg)
Red 0.128 19.362 8.575
Red 0.082 3.260 2.284

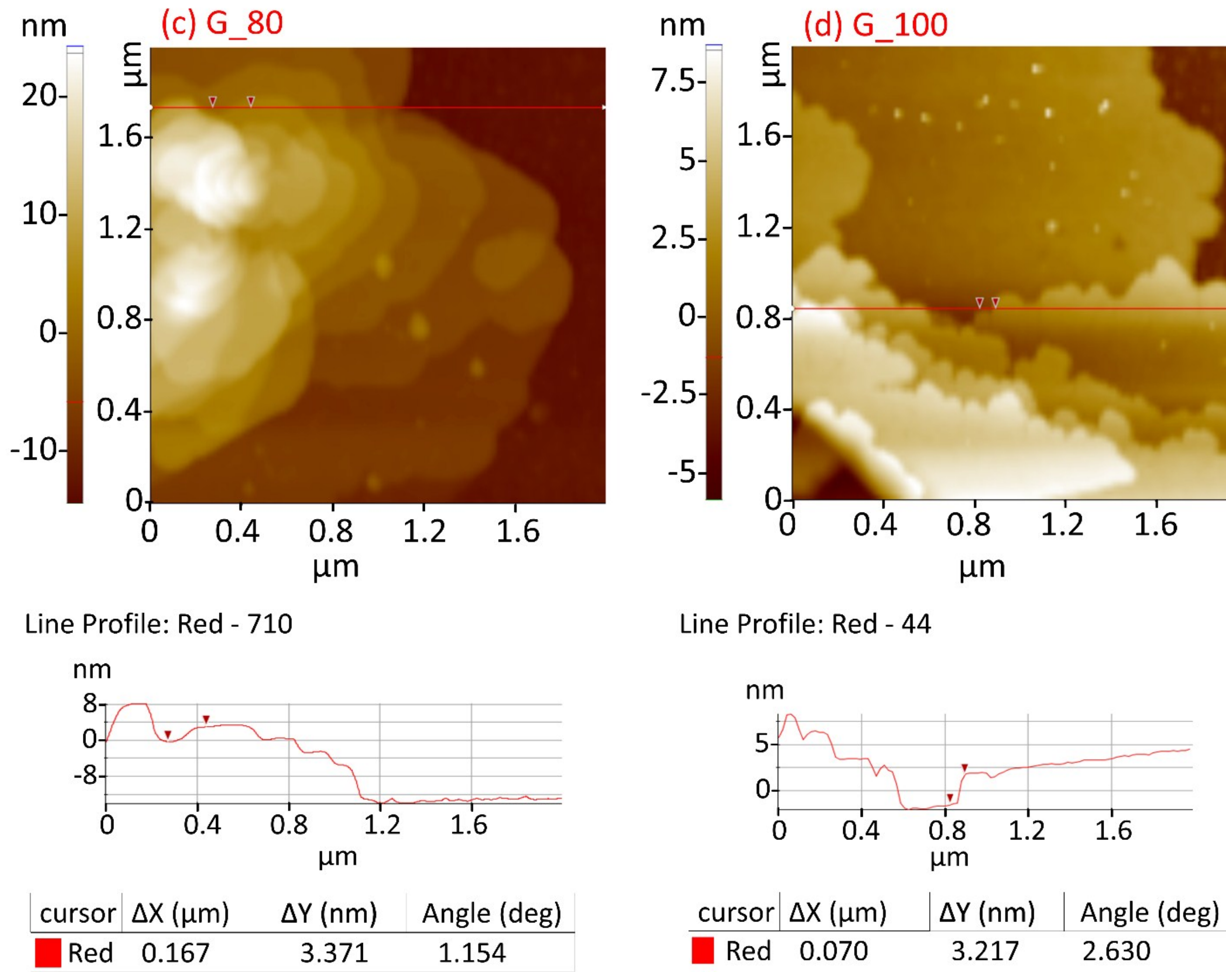
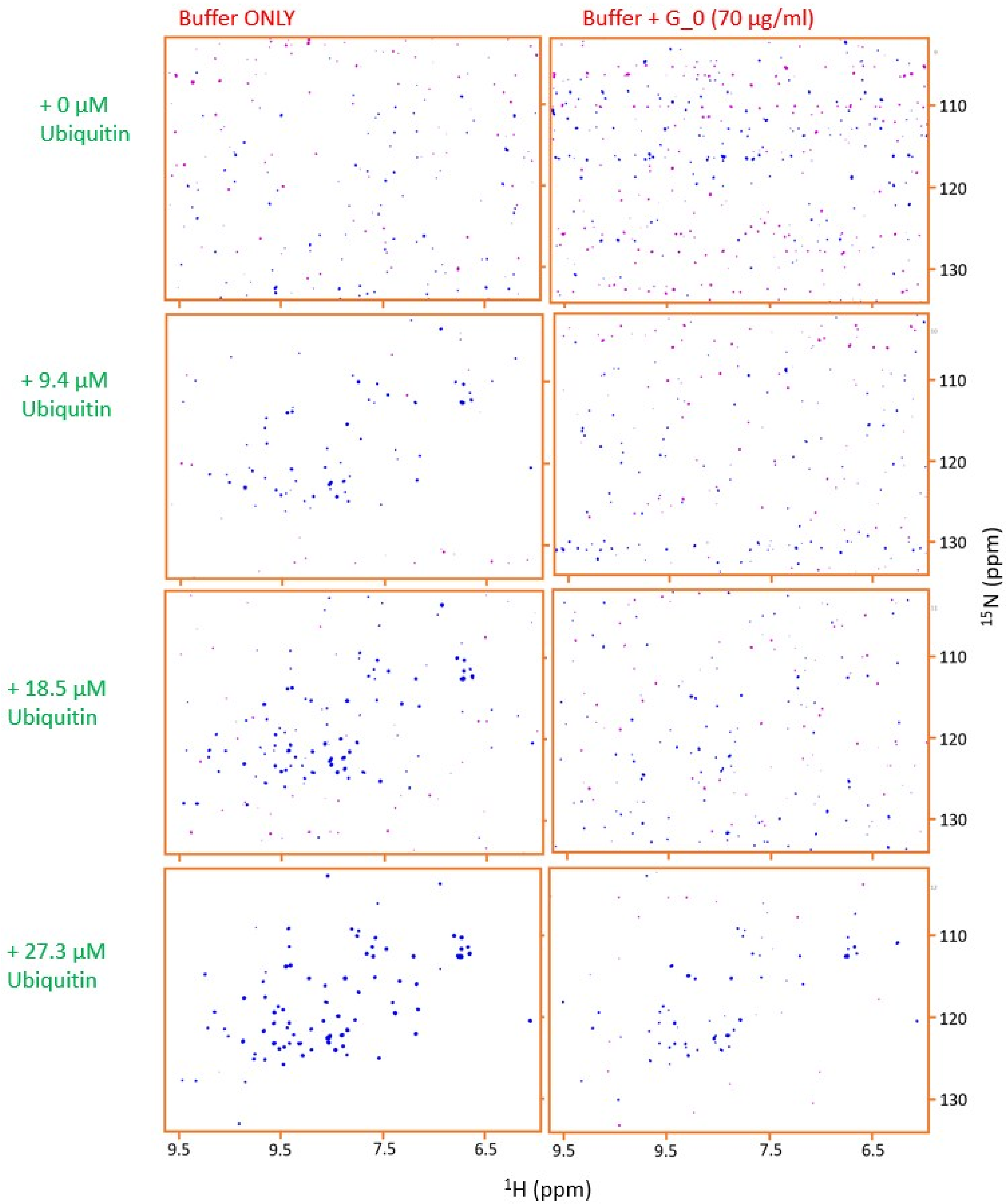


Figure S6: AFM images of different GO samples (a) G_0, (b) G_30, (c) G_80 and (d) G_100.

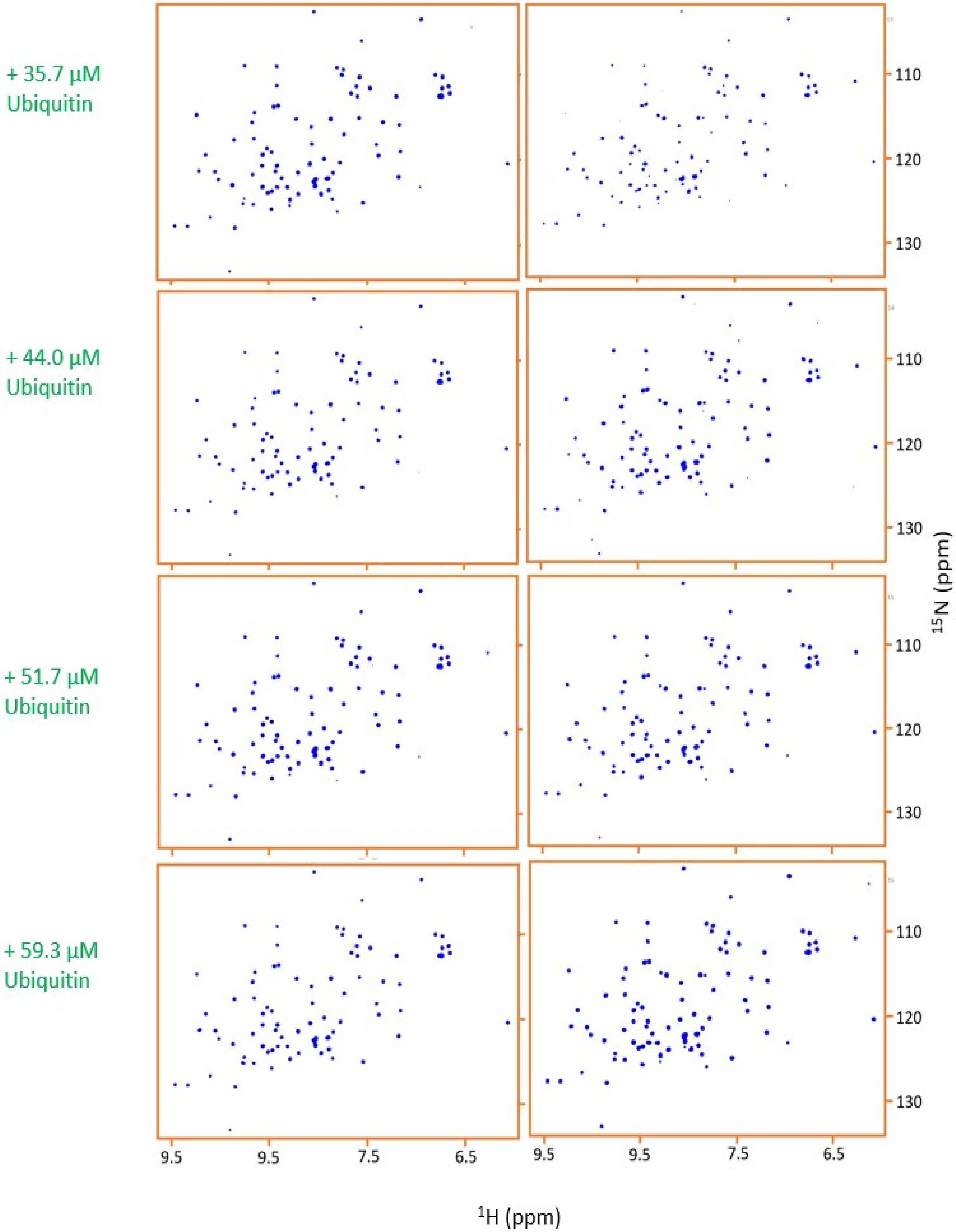
Table S1: List of the various properties of the synthesized GOs.

	G_0	G_30	G_80	G_100
Defect density	2.68	2.23	2.29	2.22
Degree of oxidation	64.7	94.6	87.0	95.2
ζ in mV (in water)	-4.65	-34.67	-24.13	-34.23
ζ in mV (in buffer)	-27.4	-33.4	-30.4	-37.1
sp^2/sp^3	0.36	0.05	0.15	0.05
d-spacing (A°)	9.3	9.6	9.8	9.6
C/O ratio	1	0.55	0.88	1.03

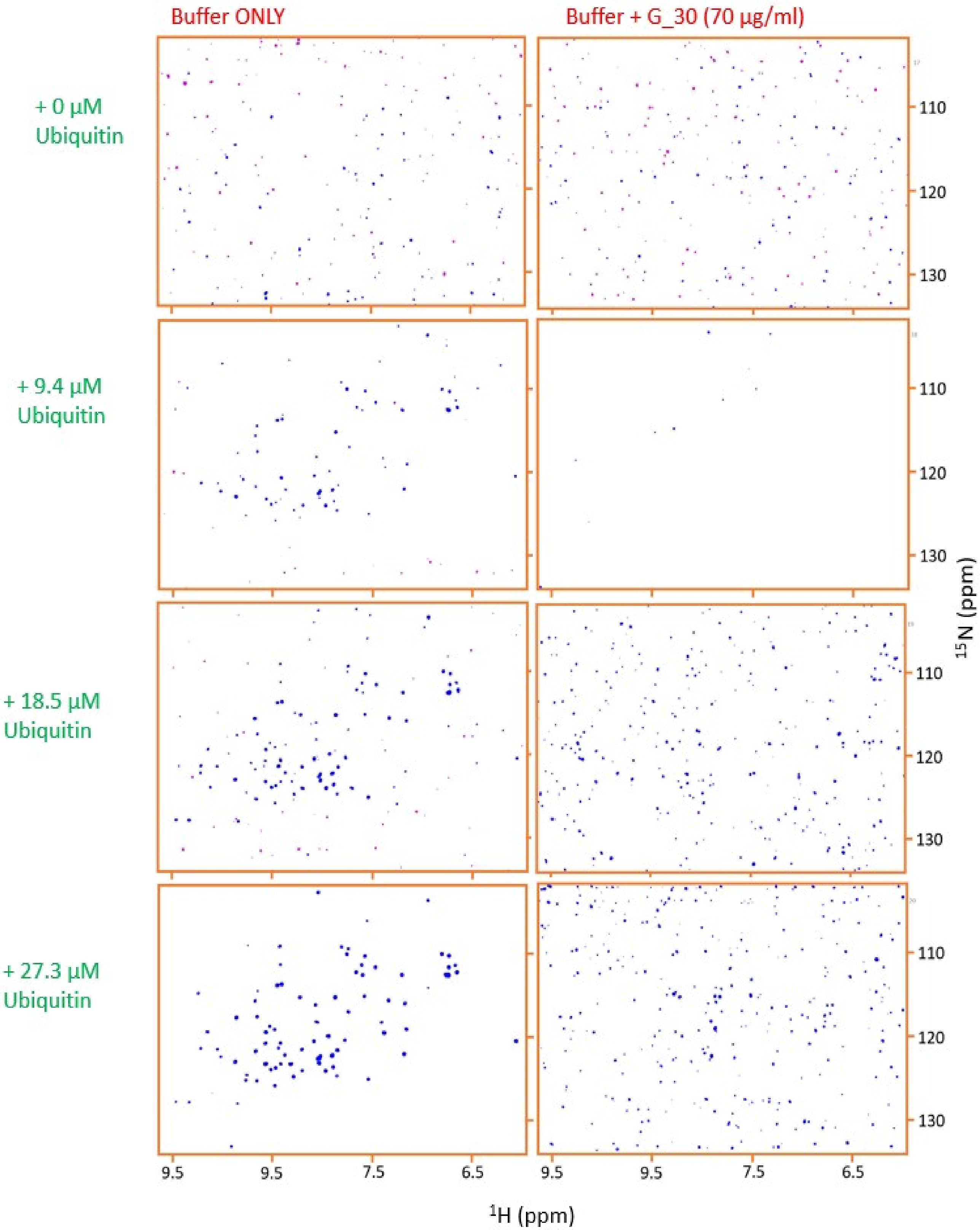
All the GO samples were bath sonicated for 15 mins. When dispersed in water, the samples showed considerable difference in the zeta potential values. While G_0 showed a zeta potential of about -4.65 mV, G _80 showed a potential of -24.13 mV, G_30 and G_100 showed zeta potential of -34.67 and -34.13 respectively. On dispersing the samples in phosphate buffer with pH 6, all the samples exhibited good dispersivity. G_0, G_30, G_80, G_100 showed values of -27.4, -33.4, -30.4 and -37.1 respectively, indicating that all the samples had good colloidal stability at pH 6 considering their zeta potential values when dispersed in water with pH 7; the latter three already were stable as colloidal dispersions in water. The reason behind all the GO showing better stability at pH 6 can be contributed to the factor that pKa of GO lies between 4.3 to 6. Hence, the GO samples are more stable in the comparatively acidic environment.



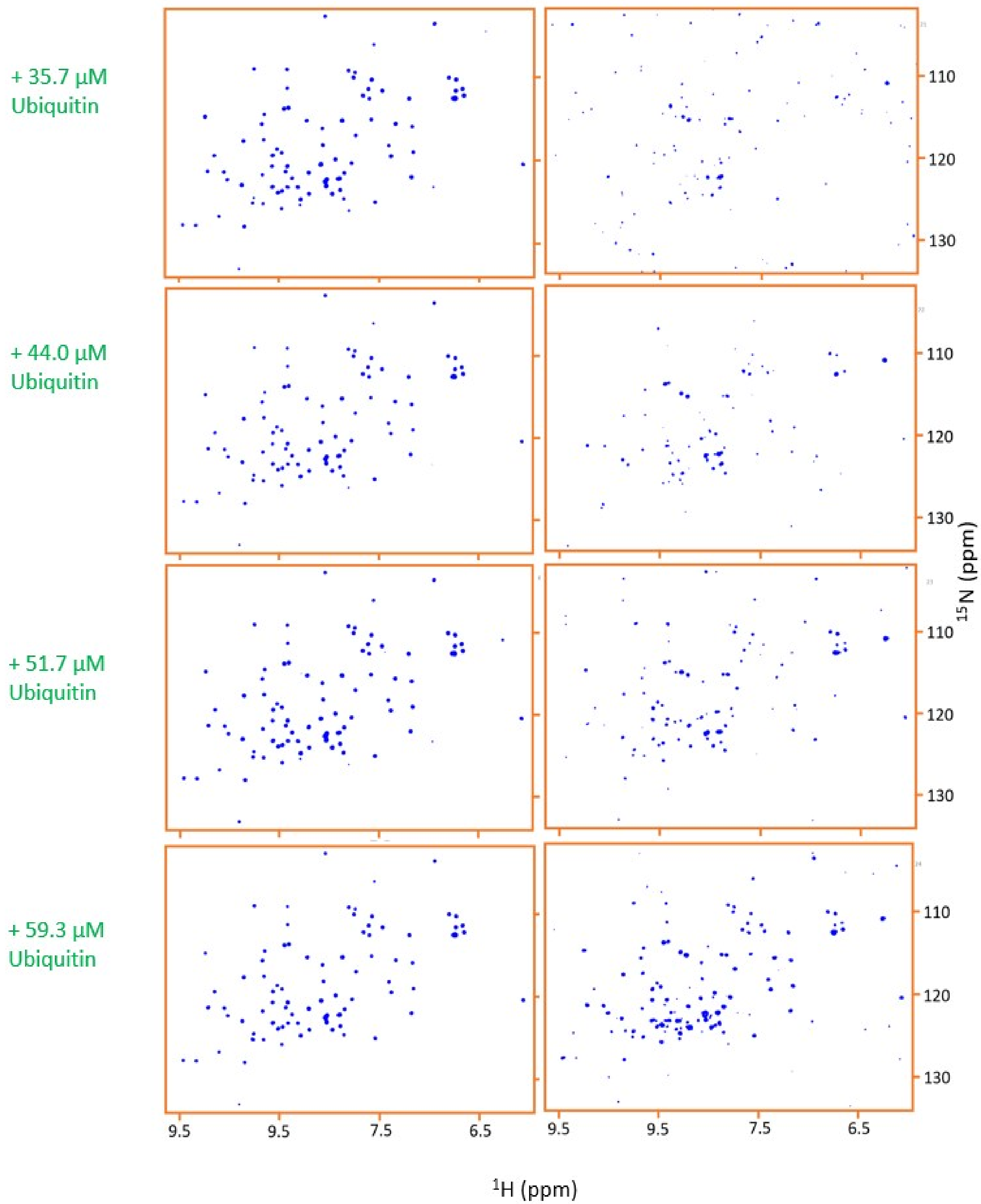
Buffer ONLY
Buffer + G_0 (70 µg/ml)
+ 0 µM Ubiquitin
+ 9.4 µM Ubiquitin
+ 18.5 µM Ubiquitin
+ 27.3 µM Ubiquitin
110
120
130
^{15}N (ppm)
9.5
9.5
7.5
6.5
^{1}H (ppm)



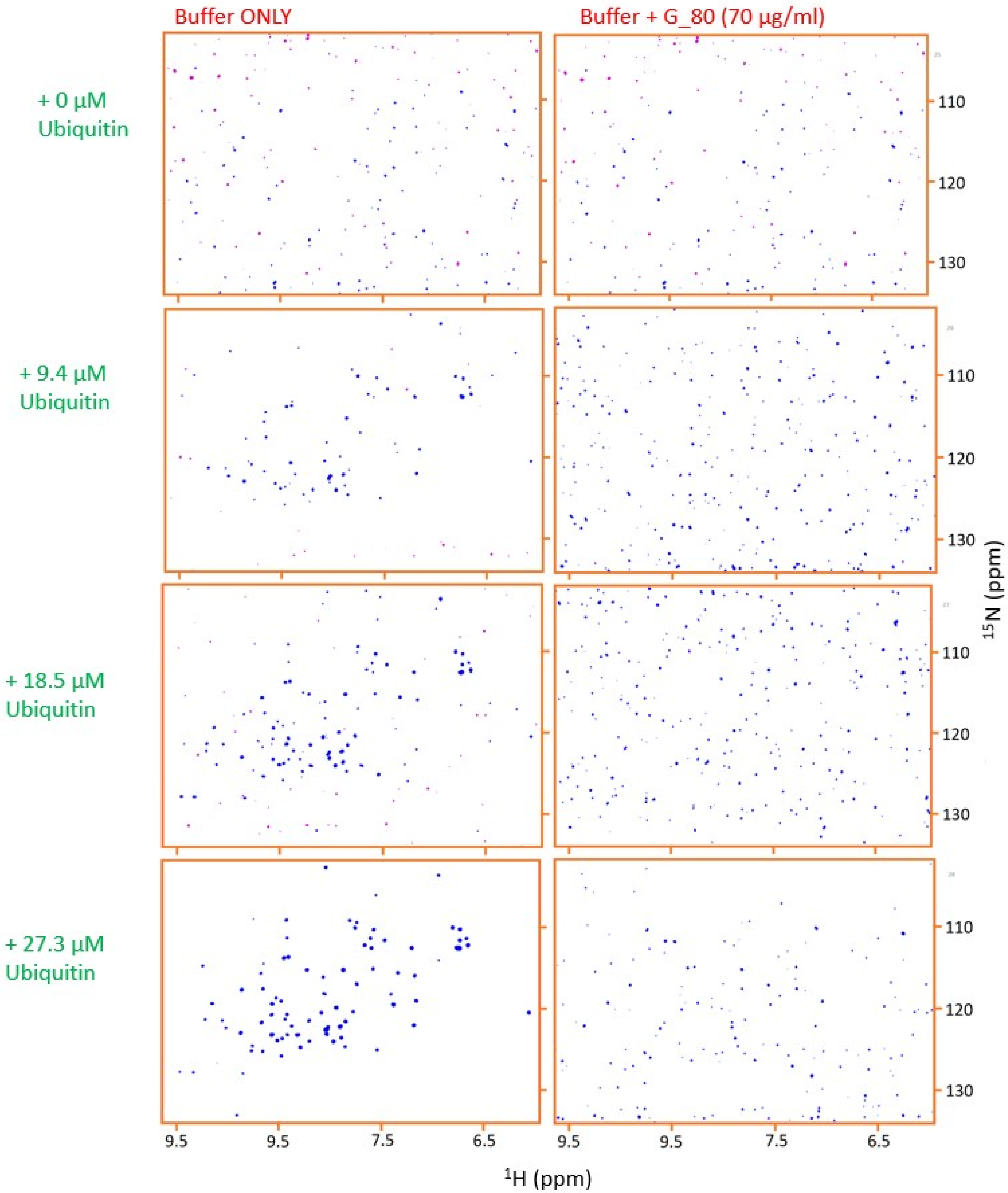
+ 35.7 µM Ubiquitin
+ 44.0 µM Ubiquitin
+ 51.7 µM Ubiquitin
+ 59.3 µM Ubiquitin
110
120
130
9.5
7.5
6.5
^{15}N (ppm)
^{1}H (ppm)

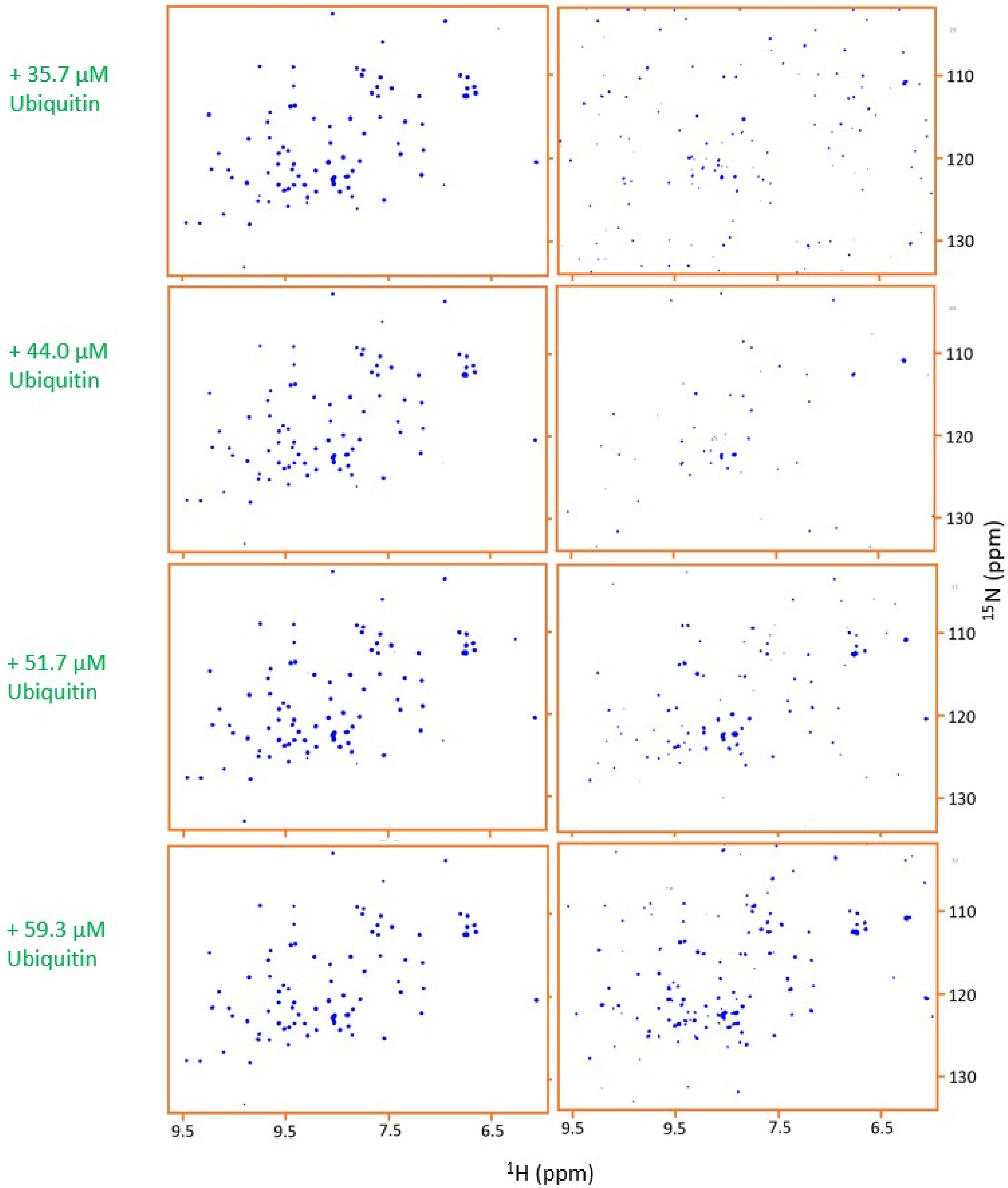


Buffer ONLY
Buffer + G_30 (70 µg/ml)
+ 0 µM Ubiquitin
+ 9.4 µM Ubiquitin
+ 18.5 µM Ubiquitin
+ 27.3 µM Ubiquitin
110
120
130
15N (ppm)
9.5
7.5
6.5
1H (ppm)

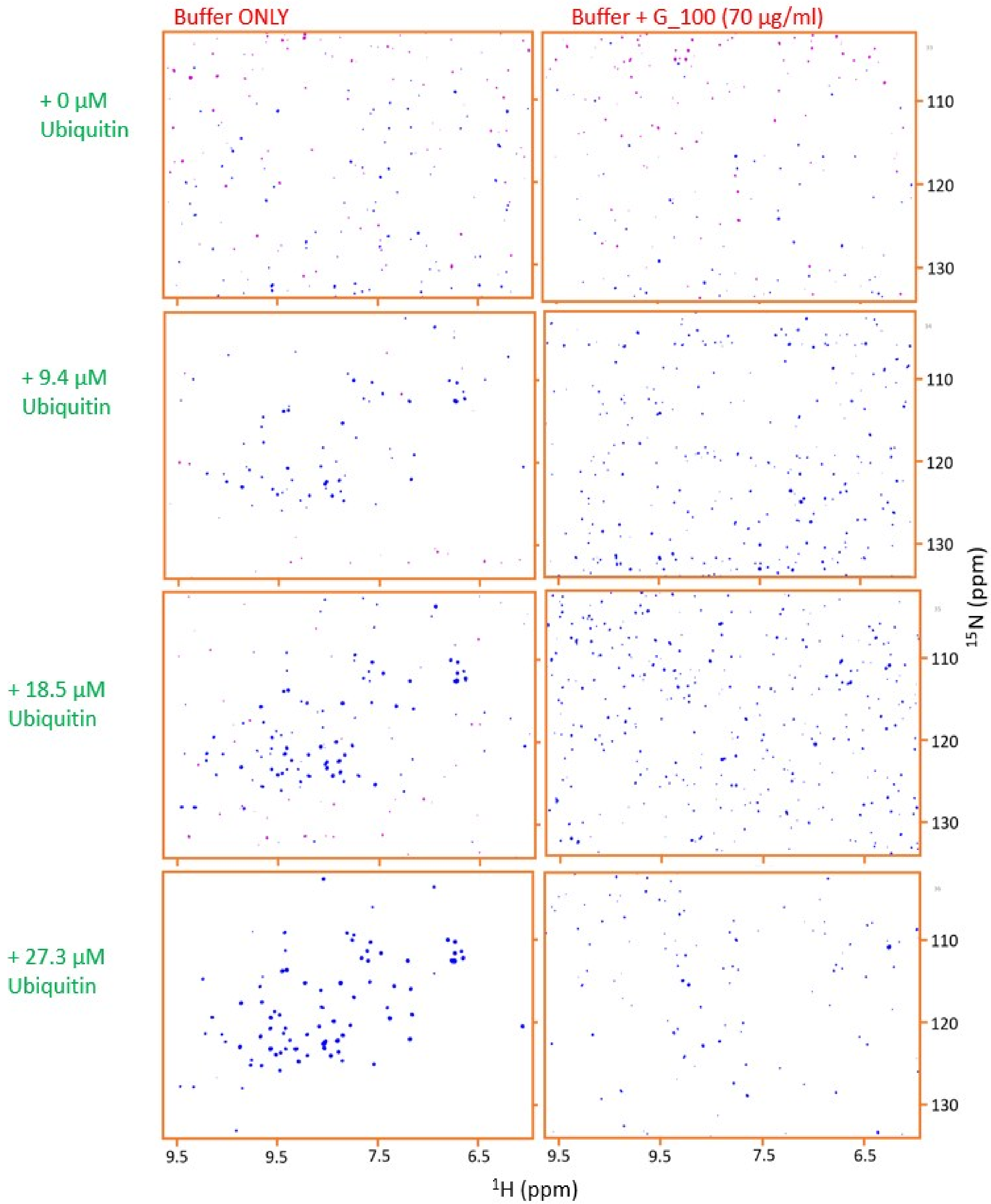


+ 35.7 µM
Ubiquitin
+ 44.0 µM
Ubiquitin
+ 51.7 µM
Ubiquitin
+ 59.3 µM
Ubiquitin
110
120
130
9.5
7.5
6.5
^{15}N (ppm)
^{1}H (ppm)


Buffer ONLY
Buffer + G_80 (70 µg/ml)
+ 0 µM Ubiquitin
+ 9.4 µM Ubiquitin
+ 18.5 µM Ubiquitin
+ 27.3 µM Ubiquitin
110
120
130
9.5
9.5
7.5
6.5
¹H (ppm)
¹⁵N (ppm)



+ 35.7 µM Ubiquitin
+ 44.0 µM Ubiquitin
+ 51.7 µM Ubiquitin
+ 59.3 µM Ubiquitin
110
120
130
9.5
9.5
7.5
6.5
^{1}H (ppm)
^{15}N (ppm)



Buffer ONLY
Buffer + G_100 (70 µg/ml)
+ 0 µM Ubiquitin
+ 9.4 µM Ubiquitin
+ 18.5 µM Ubiquitin
+ 27.3 µM Ubiquitin
110
120
130
15N (ppm)
9.5
9.5
7.5
6.5
1H (ppm)

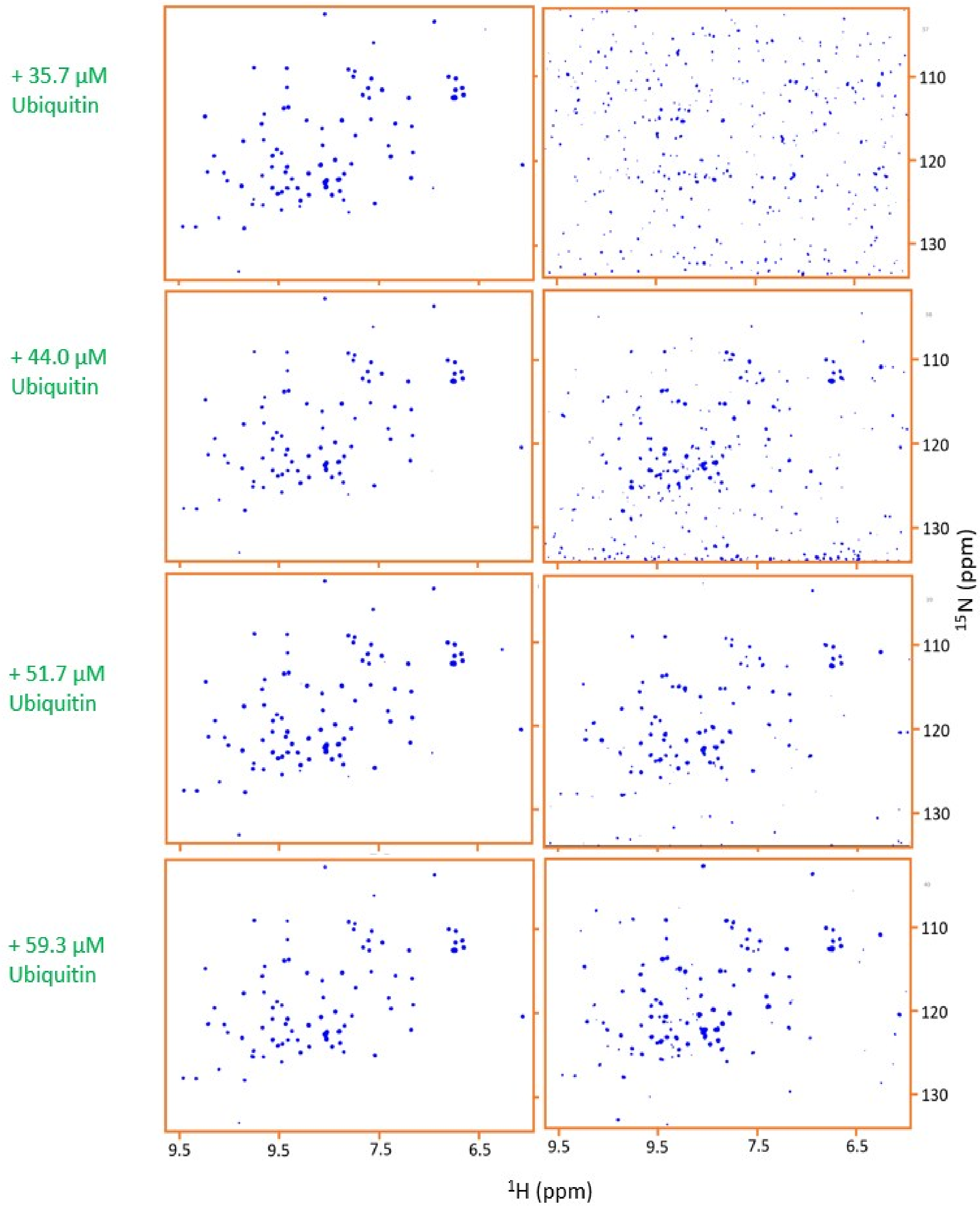


Figure S7: Reverse titrations. 2D [^{15}N, ^{1}H] HSQC spectra of free ubiquitin (left column) at different concentrations of protein (as indicated on the left) and 2D [^{15}N, ^{1}H] HSQC spectra of GO-Ubq conjugates containing 70 µg/ml each GO (as indicated on the top of each column) at the same protein concentrations as for the free protein.

Theoretical calculation of the amount of bound protein to each GO sample from the reverse titration.

For G_0: taking 27.3 µM the maximum concentration (because after this protein concentration all protein peaks start appearing in the conjugate) that can be bound to G_0:

70 µg/ml of G_0 binds ~ 27.3 µM of Ubq

1 mM of Ubq corresponds to 8.6 mg in 1 ml

Therefore, 27.3 µM of Ubq corresponds to = (8.6/1000) x 27.3 mg = 0.23 mg

Thus, loading capacity of the G_0 is ≈ (0.2/70) mg ≅ 3 µg of protein/µg of G_0.

Similarly,

> Loading capacity of the G_30 is ≈ (0.4/70) mg ≅ 5.7 µg of protein/µg of G_30 (taking 44 µM of ubq as max. bound concentration).
>
> Loading capacity of the G_80 is ≈ (0.3/70) mg ≅ 4.3 µg of protein/µg of G_80 (taking 35.7 µM of ubq as max. bound concentration).
>
> Loading capacity of the G_100 is ≈ (0.4/70) mg ≅ 5.7 µg of protein/µg of G_100 (taking 44 µM of ubq as max. bound concentration).

Quantification of the protein loaded onto each GO sample using BCA assay:

The approximate quantification of the protein loaded to each GO was done by using BCA assay. Briefly, the GO samples (70 µg per mL of phosphate buffer at pH 6) were incubated with the ubiquitin for about 20 minutes at room temperature and followed by the centrifugation at 4 °C and 8,000 rpm for 10 minutes, we re-dispersed the GO samples in the BCA assay buffer (50:1 vol ratio of BCA solution and copper sulphate as described earlier[1]). The respective absorbance values of the GO-protein conjugates were fitted to the following equation to get the final protein bound to each GO:

[protein] in µg = 0.02634 * Absorbance + 0.02255

Which yielded:

Loading capacity of the G_0 is ≈ 3.3 µg of protein/µg of G_0.

Loading capacity of the G_30 is ≈ 4.4 µg of protein/µg of G_30

Loading capacity of the G_80 is ≈ 3.8 µg of protein/µg of G_80

Loading capacity of the G_100 is ≈ 4.6 µg of protein/µg of G_100

The measured T_2 (^1H_N) values for the free and the G_0-bound Ubiquitin at different indicated concentrations of the G_0.

			G_0			
			T_2 (^1H_N) (in ms)			
Residue number	**Free Ubiquitin**	**with 38.4 µg/ml**	**with 56.6 µg/ml**	**with 74.1 µg/ml**	**with 90.9 µg/ml**	**with 107.1 µg/ml**
1						
2	41.4±3.2	31.9±1.7	31.3±1.6	28.8±1.3	26.8±1.1	23.8±0.8
3	27±1.1	21.6±0.6	19.9±0.5	16.9±0.3	16.3±0.3	12.4±0.1
4	28.2±1.2	22.7±0.7	21.9±0.6	17.3±0.4	13.5±0.2	11±0.1
5	41.8±3.2	32.6±1.8	19.5±0.5	19.5±0.5	17.7±0.3	13.3±0.1
6	36.7±2.4	20.5±0.5	18.3±0.4	17.2±0.3	18±0.4	12.1±0.1
7	27.2±1.1	24±0.8	16.9±0.3	12.8±0.1	9±0	9.8±0.1
8	36.5±2.3	29.6±1.4	27.6±1.2	25.8±1	25±0.9	23.5±0.8
9	22±0.6	14.9±0.2	10.4±0.1	7.8±0	2.7±0.3	
10	38.8±2.7	27.9±1.2	25.5±0.9	17.6±0.3	22.7±0.7	20.8±0.5
11	37.4±2.5	24.3±0.8	22.6±0.7	20.5±0.5	18.4±0.4	16.1±0.3
12	22.3±0.7	19.7±0.5	17.9±0.4	14.6±0.2	13.5±0.2	12±0.1
13	29.9±1.4	23.4±0.7	22.4±0.7	17±0.9	13.8±0.2	9.4±0

14	42.1±3.3	29.3±1.4	27.6±1.2	24.6±0.9	22.8±0.7	19.9±0.5
15	36.6±2.3	25.2±0.9	22.7±0.7	19.7±0.5	16.5±0.3	14.3±0.2
16	31.1±1.6	25.1±0.9	24.1±0.8	20±0.5	17.2±0.3	16.2±0.3
17	32.8±1.8	25.2±0.9	23.8±0.8	21.2±0.6	19.9±0.5	18.5±0.4
18	28.21.2	22.8±0.7	21.6±0.6	18.7±0.4	17.1±0.3	15.3±0.2
19						
20	37.3±2.5	27.1±1.1	26.3±1	25.2±0.9	24.6±0.9	22.6±0.7
21	30.2±1.5	25±0.9	24.2±0.8	23.2±0.7	21.7±0.6	19.8±0.5
22	29.1±1.3	24.2±0.8	24±0.8	22.2±0.7	22±0.6	15.1±0.2
23	28.3±1.2	22.8±0.7	21.6±0.6	17.5±0.3	16.4±0.3	14.3±0.2
24						
25	60.3±7.6	42.7±3.4	39.8±2.9	32.9±1.8	28±1.2	25±0.9
26	32.1±1.7	25.9±1	24.5±0.9	21±0.6	17.6±0.3	16.2±0.3
27	29.7±1.4	22.9±0.7	21.7±0.6	20.6±0.5	17.9±0.4	12.8±0.1
28	47.7±4.4	36.5±2.3	33.6±1.9	31.7±1.6	28.8±1.3	25.7±1
29	44±3.7	30.7±1.5	28.6±1.3	26.6±1.1	23.8±0.8	21.7±0.6
30	28.2±1.2	22±0.6	21.5±0.6	19.5±0.5	16.8±0.3	13.9±0.2
31	34.3±2	27.7±1.2	26.1±1	23±0.7	22.4±0.7	20.4±0.5
32	40.1±2.9	28.5±1.3	27.4±1.1	18.9±0.4	24.5±0.8	21.7±0.6

33	34.3±2	30.2±1.5	24.3±0.8	23.3±0.7	20.3±0.5	18.6±0.4
34	24.7±0.9	19.3±0.4	15.6±0.2	13.8±0.2	12.6±0.1	8.5±0
35	80.5±14	55.6±6.3	49.9±4.9	45.1±3.9	36.2±2.3	30.1±1.5
36	32.9±1.8	23.3±0.7	22.1±0.6	21.4±0.6	20.3±0.5	18.9±0.4
37						
38						
39	69.4±10	54.2±5.9	53.2±5.7	49±4.7	48.2±4.5	44.4±3.7
40	30.8±1.5	27.1±1.1	22.8±0.7	22.7±0.7	16.8±0.3	11.1±0.1
41	31.6±1.6	24.6±0.9	23.3±0.7	17.8±0.3	16.1±0.3	12.9±0.1
42	25.7±1	20.3±0.5	18.5±0.4	17±0.3	15.9±0.3	15.3±0.2
43	16.5±0.3	12.7±0.1	10.9±0.1	8.6±0	7.3±0	5.8±0
44	23.4±0.8	20.8±0.5	18.1±0.4	16.4±0.3	15.6±0.2	14.2±0.2
45	22.5±0.7	19.8±0.5	18±0.4	14.3±0.2	13.2±0.1	7.9±0
46	30.4±1.5	21.5±0.6	16.3±0.3	4.8±0		
47	39.3±2.8	28.7±1.3	20.9±0.5	17.7±0.3	15.7±0.2	13±0.1
48	50.2±5	37.7±2.5	35.5±2.2	32.8±1.8	31.9±1.7	31.4±1.6
49	40.2±2.9	34.7±2.1	32±1.7	28.3±1.2	25.8±1	24.7±0.9
50	30.6±1.5	24.5±0.8	23.9±0.8	21.7±0.6	19.7±0.5	12.5±0.1
51	28.7±1.3	21.4±0.6	18.3±0.4	17.4±0.3	15.7±0.2	15.6±0.2

52	38.7±2.7	30.7±1.5	29.5±1.4	27.3±1.1	25.8±1	22.6±0.7
53						
54	29.6±1.4	23.2±0.7	21.3±0.6	19.9±0.5	16.3±0.3	11±0.1
55	22.9±0.7	17.4±0.3	14.4±0.2	12.1±0.1	9.6±0	7.1±0
56	39.3±2.8	30.3±1.5	25.3±0.9	23.6±0.8	20.5±0.5	16.1±0.3
57	86.3±17	65.6±9.1	60.4±7.6	60.4±7.6	55.7±6.3	47.8±4.4
58	36.6±2.4	27±1.1	25.5±0.9	24±0.8	22.2±0.6	20±0.5
59	28.9±1.3	22.3±0.7	21±0.6	17.8±0.3	13.4±0.1	11.9±0.1
60	42±3.3	29.7±1.4	27.7±1.2	25±0.9	22.8±0.7	21.6±0.6
61	28.2±1.2	20.2±0.5	19.6±0.5	17.2±0.3	16.4±0.3	10.2±0.1
62	31.2±1.6	24±0.8	22.4±0.7	21.7±0.6	20.4±0.5	19.9±0.5
63	46±4.1	35.3±2.2	32.5±1.8	29.8±1.4	26.9±1.1	24.5±0.9
64	36.9±2.4	26.5±1	22.1±0.6	19.2±0.4	17±0.3	13.6±0.2
65	27±1.1	23.1±0.7	19.7±0.5	19.3±0.4	18.1±0.4	16.2±0.3
66	25.3±0.9	19.7±0.5	19.3±0.4	15.9±0.3	6.2±0	
67	23.2±0.7	16.2±0.3	15±0.2	13.2±0.1	10.8±0.1	8.2±0
68	30.3±1.5	24.6±0.9	20.3±0.5	17.9±0.4	15.2±0.2	12.1±0.1
69	32.1±1.7	28.3±1.2	23.7±0.8	22±0.6	20.7±0.5	19.7±0.5
70	39.8±2.9	24.3±0.8	20.7±0.5	18±0.4	17±0.3	12.8±0.1

71	32.7±1.8	25.8±1	23.2±0.7	20.8±0.5	19.5±0.5	17.2±0.3
72	39.5±2.8	30.4±1.5	28.1±1.2	27.4±1.1	25.4±0.9	25.1±0.9
73	38.92±2.7	29.6±1.4	27.1±1.1	25.6±1	25.1±0.9	23.1±0.7
74	41.14±3.1	31.5±1.6	29±1.3	28.2±1.2	26.7±1.1	26.1±1
75	29.52±1.4	23.9±0.8	22.3±0.7	21.4±0.6	20.5±0.5	18.8±0.4
76	66.84±0.9	54±0.9	53.9±5.9	48.5±4.6	45.8±4	44.4±3.7

The measured T_2 (^1H_N) values for the free and the G_30-bound Ubiquitin at different indicated concentrations of the G_30.

			G_30			
			T_2 (^1H_N) (in ms)			
Residue number	**Free Ubiquitin**	**with 38.4 µg/ml**	**with 56.6 µg/ml**	**with 74.1 µg/ml**	**with 90.9 µg/ml**	**with 107.1 µg/ml**
1						
2	41.4±3.2	29.8±3.1	28.9±1.4	27.1±2.9	25.7±0.6	22.3±0.1
3	27±1.1	20.9±1.8	16.4±0.4	15.4±0.6	11.3±0.1	7.4±0.8
4	28.2±1.2	19.9±2	19.3±0.9	18.3±0.2	18.2±0.2	17.9±0.1
5	41.8±3.2	29.2±1.6	26.7±1.6	21.3±0.2	18±0.4	16.3±0.6
6	36.7±2.4	24.6±0.6	20.8±0.5	12.6±0.2	8.5±0.2	
7	27.2±1.1	20.5±0.9	17.2±0.1	15.3±0.3	12.3±0.2	6.1±0.1
8	36.5±2.3	25.5±1.5	22.7±0	21.2±1	20±0.2	19.7±1
9	22±0.6	15.4±1.8	14.3±0.2		7.3±0.3	6.4±0.3
10	38.8±2.7	25.2±2.4	22.9±0.2	16.7±0.3	9.1±0.3	4.6±0.2

11	37.4±2.5	25.8±1.1	20.9±0.3	16.8±0.2	14.9±0.2	12.3±0.3
12	22.3±0.7	16.3±4.4	15.2±0.8	10.9±0.4	10.8±0.3	8.3±0.6
13	29.9±1.4	19.5±2.4	16.2±0.1		7.2±0.2	3.3±0.2
14	42.1±3.3	29.9±2.2	26.1±0.7	24±0.6	18.9±0.2	18.4±0.6
15	36.6±2.3	27.8±1.1	21.2±1	15.4±0.1	9.5±0.3	
16	31.1±1.6	24.6±2.7	22.1±0.2	20.5±0.4	18.4±0.2	16.2±0.3
17	32.8±1.8	23.3±2.2	21.7±0.4	17.1±0.3	15.8±0.2	
18	28.21.2	21.4±1.1	19.4±0.1		13.8±0.4	
19						
20	37.3±2.5	29.1±2	28.4±1.2	24.3±0.3	19.2±0.2	17.7±0.1
21	30.2±1.5	22.9±1.1	20.8±1.1	18.7±0.7	18.3±0.3	16.5±0.2
22	29.1±1.3	22.7±2	19.5±0.5	16.9±1	13.4±0.5	5.2±0.2
23	28.3±1.2	19±5.2	17.5±0.7	14.7±0.3	9.2±0.1	4.2±0.3
24						
25	60.3±7.6	44.1±4.4	41±0.8	31.4±3.2	28.4±1	21.7±0.2
26	32.1±1.7	25±2	23.4±0.4	21.8±0.8	19.6±0.4	16.7±0.3
27	29.7±1.4	22.2±0.7	17.5±0.2	13.7±0.5	9.8±0.3	5.1±0.1
28	47.7±4.4	37.7±2.7	34.3±3.2	31.9±0.5	28.6±2.6	24.8±0.3

29	44±3.7	36.2±0.6	30.4±1.1	25.5±1	20.7±1	9.8±0.1
30	28.2±1.2	22±2	19.5±1.1	17.5±0.7	13.6±0.2	10.4±0.6
31	34.3±2	25.4±0.6	20.9±0.4	18.5±0.1	15.8±0.5	12.7±0.6
32	40.1±2.9	30.9±1.5	28.9±3.2	26.5±0.4	20.5±0.2	18.9±1
33	34.3±2	24.7±0.3	24±1.6	21.2±0	18.2±0.6	13±0.2
34	24.7±0.9	14±0.5	9±0.3	6.4±0.3	3.6±0.1	3±0.5
35	80.5±14	46.1±0.7	41.6±2.5	36.6±3	10.8±0.7	
36	32.9±1.8	23.7±0.3	20.1±0.4	17.5±0.6	12.7±0.3	4±0.7
37						
38						
39	69.4±10	55.2±0.3	45.1±2.4	36.1±0.3	31.9±4.5	25.7±0.6
40	30.8±1.5	21.3±0.1	17.9±1	15.7±0.2	12.6±0.5	10.5±0.1
41	31.6±1.6	23.4±0.6	19.3±0.4	15.5±0.4	13.3±0.1	11.4±0.2
42	25.7±1	17.3±0.6	17.2±0.5	12.7±0.6	11.6±0.2	8.2±0.5
43	16.5±0.3	9.4±0.5	7.7±0.7		2.3±0.2	
44	23.4±0.8	17.1±0.4	16.2±0.1	14.5±0.7	14±0.2	12.6±0.9
45	22.5±0.7	15±0.5	12.8±0.6	10.1±0.2	5.2±0.2	
46	30.4±1.5	20.4±0.5	13.9±0.3			

47	39.3±2.8	30.3±0.1	27.4±1.3	25.1±1.4	23±1.1	20.9±0.6
48	50.2±5	40.7±4.7	39.6±2.5	38.5±1.4	33±0.3	32.4±1.9
49	40.2±2.9	32.4±4.5	31.6±2.4	30±0.8	27.1±0.6	25.6±1.4
50	30.6±1.5	20.8±0.8	15.6±1.7	10.6±0.3	7.2±0.4	
51	28.7±1.3	19.4±0.6	18.4±1.6		12.4±0.3	11.9±0.7
52	38.7±2.7	30.2±2	26.7±1.1	24.8±0	19.8±1.7	16.7±0.3
53						
54	29.6±1.4	23.1±2	19.9±0.5	15.4±0.3	14.2±0.2	10.7±0.2
55	22.9±0.7	16.5±3.2	13.9±0.4		8.2±0.2	7.1±0.1
56	39.3±2.8	31.5±3.6	27.1±1.1	21.2±0.1	19.3±0.4	14.6±0.6
57	86.3±17	66.4±14.8	58.4±6.6	53.1±5.4	47±0.1	42.6±5.6
58	36.6±2.4	27.1±0.6	26±2.2	24.9±0.8	21±0.7	20.8±0.5
59	28.9±1.3	19.7±0.8	18.5±1.6	16.2±0.3	11.7±0.3	9.3±0.7
60	42±3.3	27.9±4.2	25.6±0.4	19.3±0.5	16.1±0.3	
61	28.2±1.2	19.5±1.1	18.1±0.2	15.2±0.1	13.9±0.5	10.7±0.2
62	31.2±1.6	20.3±2.3	16.2±0.3	15.6±0.9	13.1±0.1	9.4±0.2
63	46±4.1	35.5±1.5	30.4±3.5	23.9±0.3	21.6±1	18±0.6
64	36.9±2.4	23.1±0.9	20.7±3.5	13.5±0.4	9±0.4	

65	27±1.1	21±2	17.8±3.5	15.9±1.6	12.5±0.6	11.2±0.8
66	25.3±0.9	16.7±3.5	14.7±1	11.4±0.2	9.2±0.3	8.1±0.7
67	23.2±0.7	15±2.1	13.3±0.6		5.6±0.4	
68	30.3±1.5	20.3±5.3	17.1±0.4	13.8±0.3	7.2±0.1	
69	32.1±1.7	22.6±1.8	19.6±0.4	17.6±0.2	15.8±0.5	14±0.7
70	39.8±2.9	20.3±1.6	16.2±0.4	12.3±0.7	5.1±0.1	
71	32.7±1.8	25.5±2	21.3±2.4	17±0.3	16.1±0.5	14.2±0.5
72	39.5±2.8	28.6±3.6	24.5±0.7	23.7±2.6	21.7±1.8	20.3±2.2
73	38.92±2.7	27.8±2.7	24.1±6.5	23.1±2	21.1±1.1	20.9±9.6
74	41.14±3.1	32.1±2	29.6±3.2	29.1±2	28.6±1.4	26.7±2.3
75	29.52±1.4	22.7±1.5	20.6±1.4	19.5±0.3	17.3±1.4	15.8±0.8
76	66.84±0.9	53.5±3.6	50.8±1.1	50.7±8.5	47.2±2	45.8±0.9

The measured T_2 (^1H_N) values for the free and the G_80-bound Ubiquitin at different indicated concentrations of the G_80.

			G_80			
			T_2 (^1H_N) (in ms)			
Residue number	Free Ubiquitin	with 38.4 µg/ml	with 56.6 µg/ml	with 74.1 µg/ml	with 90.9 µg/ml	with 107.1 µg/ml
1						
2	41.4±3.2	29.8±0.3	27.4±0.4	22.4±0.1	14.3±0.2	5.8±0.2
3	27±1.1	20.5±0.1	16.7±0.7	15.6±1	12.7±1	7.4±0.9
4	28.2±1.2	22±0.2	18.6±0.4	16.1±0.6	12.1±0.3	10.4±0.6
5	41.8±3.2	30.1±0.3	23.8±0.6	20.1±0.2	16.3±0.6	13.8±0.3
6	36.7±2.4	30.1±0.6	24.6±0.5	19.1±0.3	16.5±0.2	5±1
7	27.2±1.1	21.2±0.2	18.8±0.1	16.3±0.3	15.3±0.3	11.3±0.9
8	36.5±2.3	28.5±0.2	25.2±0.1	20.8±0.6	16.4±0.2	10.2±0.3
9	22±0.6	15.2±0.1	11±0.9	10.6±0.2	7±0.5	4±0.2
10	38.8±2.7	30.3±0.2	24.1±0.7	19.8±0.2	12±0.1	6.6±0.1
11	37.4±2.5	26.2±0.2	25.4±0.8	20.2±0.1	17.2±0.5	15.5±0.7
12	22.3±0.7	17.4±0.2	15±0.5	12.7±0.6	11.4±0.2	10±0.1

13	29.9±1.4	22.8±0.1	20.4±0.8	18.6±0.7	14.7±0.4	9.3±0.1
14	42.1±3.3	30.3±0.3	27.8±0.4	22.7±0.1	18.9±0.2	11.9±0.7
15	36.6±2.3	28.9±0.3	17.5±0.2	14.3±0.6	10.6±0.3	6.6±0.2
16	31.1±1.6	25.8±0.8	22.1±0.2	19.6±0.1	17.4±0.3	17±0.2
17	32.8±1.8	25.6±0.2	21.3±0.2	19±1	16.1±0.4	12.6±0.3
18	28.21.2	23.3±0.8	23±0.6	20.4±0.4	19.8±0.2	17.9±0.1
19						
20	37.3±2.5	29.7±0.3	27.1±0.4	23.1±0.7	20.5±0.2	16±0.3
21	30.2±1.5	24.1±0.3	23.1±0.1	21.6±0.3	19.5±0.2	18.5±0.5
22	29.1±1.3	23.5±0.4	22.3±0.1	19±0.3	15.9±0.2	14.9±0.2
23	28.3±1.2	22.1±0.2	18.1±0.2	14.7±0.3	12.2±0.3	8.8±0.1
24						
25	60.3±7.6	45.9±0.1	39.8±0.4	32.6±0.1	26±0.3	24.4±0.3
26	32.1±1.7	26.3±0.6	22.3±0.1	21.1±0.3	19.7±0.5	17.1±0.7
27	29.7±1.4	21.9±0.6	20.7±0.1	20.4±0.9	18.6±0.8	15.9±0.9
28	47.7±4.4	33.8±0.2	30±0.1	29.3±0.5	27.3±0.6	25.3±0.6
29	44±3.7	32.6±0.6	28.2±0.2	25.1±0.6	21.6±0.4	19.4±0.9
30	28.2±1.2	23.1±0.6	18.9±0.5	14.1±0.9	13±0.5	10.2±0.3

31	34.3±2	25.1±0.4	22±0.2	20.9±0.4	18.5±0.1	14.8±0.3
32	40.1±2.9	27.7±0.1	24.9±0.7	22.5±0.3	18.1±0.2	15±0.9
33	34.3±2	23.6±0.1	20.6±0.3	18.5±0.1	16.5±0.2	12.7±0.6
34	24.7±0.9	19±0.2	15.8±0.2	11.3±0.3	6±0.4	2.3±0.2
35	80.5±14	55.6±0.1	50.7±0.1	43.5±0.1	36.2±0.2	25.8±0.5
36	32.9±1.8	25.4±0.2	21.4±0.2	16.8±0.2	15.1±0.5	12.5±0.2
37						
38						
39	69.4±10	50±0.3	48.3±0.1	45.4±0.3	40.6±0.1	37.1±0.8
40	30.8±1.5	21±0.8	19.4±0.1	16±0.3	13.9±0.2	12±0.6
41	31.6±1.6	23±0.4	17.4±0.2	15.2±0.2	14.5±0.5	11.4±0.2
42	25.7±1	20.1±0.2	15.7±0.4	14.4±0.3	11.6±0.2	9.6±0.8
43	16.5±0.3	11.4±0.1	8.8±0.6	7.8±1	4.6±0.3	
44	23.4±0.8	17.8±0.1	14.8±0.1	13.4±0.6	10.8±0.5	5.5±0.6
45	22.5±0.7	15.2±0.7	13.4±0.2	12.6±0.4	7.9±0.7	0.4±0
46	30.4±1.5	20.5±0.6	14±0.5	13.1±0.3	8.5±0.3	
47	39.3±2.8	30.7±0.2	27.2±0.1	25.2±0.2	21.2±0.1	15.7±0.2
48	50.2±5	39.2±0.2	36±0.3	33.1±0.3	30.9±0.5	26.8±0.7

49	40.2±2.9	28.9±0.3	24.1±0.3	21.3±0.6	16.1±0.2	14.1±0.5
50	30.6±1.5	22±0.3	19.9±0.2	15.8±0.2	10.5±0.2	4.1±0.3
51	28.7±1.3	23.2±0.4	22±0.1	21.6±0.9	20.3±0.2	18.6±0.2
52	38.7±2.7	28.3±0.4	26.3±0.8	21.7±0.3	19.9±0.2	16.7±0.4
53						
54	29.6±1.4	22.5±0.1	19.6±0.4	15.7±0.6	13.6±0.5	9.4±0.3
55	22.9±0.7	17.6±0.2	14.6±0.2	11±0.2	7.3±0.5	6.4±0.3
56	39.3±2.8	29.9±0.1	26±0.4	22±0.3	17.7±0.2	12.6±0.5
57	86.3±17	67.3±0.2	65.7±0.1	60±0.1	55.8±0.2	46.3±0.9
58	36.6±2.4	24.9±0.8	21.6±0.2	18.7±0.2	16.9±0.5	12.8±0.5
59	28.9±1.3	21.4±0.6	18.8±0.2	14.2±0.4	10.7±0.6	3.5±0.2
60	42±3.3	31.9±0.1	27.3±0.2	22.7±0.1	20.6±0.4	11.9±0.5
61	28.2±1.2	21.5±0.1	17.8±0.1	15.5±0.2	13±0.5	8.6±0.3
62	31.2±1.6	22.8±0.4	21.5±0.1	19±0.4	12.8±0.5	10.9±0.5
63	46±4.1	34.1±0.6	30.4±0.4	26.7±1	23.7±0.2	19.9±0.4
64	36.9±2.4	26.2±0.2	23.6±0.2	20.3±0.2	15.9±0.3	5.6±0.1
65	27±1.1	21.8±0.5	18.9±0.2	17.3±0.2	15.4±0.6	11.3±0.1
66	25.3±0.9	18.2±0.3	16.4±0.2	14.9±0.2	11.3±0.2	7.2±0.6

67	23.2±0.7	17.8±0.2	15.3±0.4	8.2±0.8	3.7±0.3	2.8±0.2
68	30.3±1.5	23.3±0.2	19.1±0.1	17±0.3	14.8±0.4	4.3±0.2
69	32.1±1.7	26.3±0.6	22.8±0.2	21.8±0.1	20.6±0.2	15.2±0.1
70	39.8±2.9	29.8±0.8	25.5±0.2	21.1±0.1	18.7±1	8.1±0.5
71	32.7±1.8	25.8±0.3	25±0.1	23.4±0.3	21.2±0.2	20.8±0.1
72	39.5±2.8	30±0.1	26.4±0.5	24.5±0.7	20.9±0.6	19.5±0.6
73	38.92±2.7	30.4±0.2	27.9±0.3	25.5±0.3	25.1±0.2	20.7±0.7
74	41.14±3.1	34.5±1	31.5±0.1	30.3±0.5	26.7±0.2	25.3±0.5
75	29.52±1.4	20.1±0.8	19.8±0.5	17.1±1	16.1±0.2	14.9±0.1
76	66.84±0.9	54±0.4	51.4±0.1	47.9±0.3	46.5±0.1	43.5±0.2

The measured T_2 (^1H_N) values for the free and the G_100-bound Ubiquitin at different indicated concentrations of the G_100.

			G_100 T_2 (^1H_N) in ms			
Residue number	Free Ubiquitin	with 38.4 µg/ml	with 56.6 µg/ml	with 74.1 µg/ml	with 90.9 µg/ml	with 107.1 µg/ml
1						
2	41.4±3.2	28.9±0.1	23.8±0.7	16.8±0.4	12.3±0.8	10.4±0.3
3	27±1.1	21±0.2	19.1±0.2	17.8±0.4	15.6±1	5.4±0.2
4	28.2±1.2	22±0.2	19.5±0.1	18.6±0.4	16.9±0.3	7.3±0.4
5	41.8±3.2	30.5±0.4	26.6±0.1	22.1±0.6	18.4±0.9	4.2±0.9
6	36.7±2.4	22.5±0.5	19.4±0.6	16.1±0.9	12.9±0.7	0±0
7	27.2±1.1	20.2±0.6	18.5±0.8	15.3±0.3	11.7±0.4	6.8±0.3
8	36.5±2.3	24.6±0.6	22.7±0.7	20.5±0.3	15.4±0.1	4±0.2
9	22±0.6	15.2±0.1	12.1±0.2	10.6±0.2	8.8±0.2	0±0
10	38.8±2.7	30.3±0.2	24.8±0.2	21.3±0.2	14±0.2	7.4±0.3
11	37.4±2.5	28.4±0.1	23.6±0.1	20.9±0.3	16.1±0.4	12.7±0.1

12	22.3±0.7	17.2±0.2	15.4±0.1	14.3±0.2	11.4±0.2	8.9±0.2
13	29.9±1.4	23.4±0.2	21.3±0.2	19.8±0.4	15.4±0.2	6±0.2
14	42.1±3.3	32±0.1	29±0.1	26.1±0.7	19.4±0.5	14.3±0.1
15	36.6±2.3	26.3±0.3	23.4±0.2	19.4±0.6	16.8±0.5	3.3±0.1
16	31.1±1.6	23.3±0.8	21.5±0.1	19.6±0.1	17.4±0.3	15.6±0.9
17	32.8±1.8	25.6±0.2	20.3±0.7	18.1±0.2	15±0.4	11.2±0.1
18	28.21.2	21.5±0.1	18±0.2	16.6±0.2	11.5±0.4	10.4±0.6
19		0±0	0±0	0±0	0±0	0±0
20	37.3±2.5	27.6±0.6	24.6±0.4	20.1±0.1	17.9±0.2	14.9±0.2
21	30.2±1.5	23.2±0.2	19.3±0.2	17.5±1	14.3±0.1	11.8±0.6
22	29.1±1.3	23±0.3	20.4±0.2	18.8±0.2	16.3±0.3	15.1±0.3
23	28.3±1.2	20.4±0.3	18.4±0.2	15±0.6	12.4±0.9	9.1±0
24		0±0	0±0	0±0	0±0	0±0
25	60.3±7.6	41.6±0.1	36.8±0.4	32±0.6	27.8±0.5	22.3±0.6
26	32.1±1.7	22.9±0.3	21.2±0	17.3±0.1	15.1±1	12.2±0.2
27	29.7±1.4	23.2±0.2	21.3±0.3	19.3±0.2	13.1±0.9	4.8±0.3
28	47.7±4.4	36.2±0.1	31.5±0.4	26.7±0.3	21.5±0.2	19.1±0.2
29	44±3.7	34±0.2	27.5±0.8	24.7±0.3	19.8±0.2	12.3±0.3
30	28.2±1.2	22±0.2	18.6±0.4	15.2±0.1	13.8±0.4	10.7±0.2

31	34.3±2	26.4±0.2	23.7±0.1	21.3±0.7	17.5±0.2	16.5±0.2
32	40.1±2.9	32.5±0.5	29.7±0.6	27.3±0.8	23.7±0.2	20.1±0.9
33	34.3±2	26±0.1	21.6±0.1	19.9±1	18.8±0.2	13.4±0.6
34	24.7±0.9	18.8±0.1	15.3±0.7	13.2±0.7	11.9±0.2	2.7±0.2
35	80.5±14	58±0.3	61.2±0.1	45.1±0.3	29.8±0.6	11.3±0
36	32.9±1.8	23.4±0.2	22.4±0.8	20.7±0.1	18.8±0.6	15.1±0.5
37						
38						
39	69.4±10	54.2±0.2	48.6±0.2	46.5±0.5	40.3±1	34.7±0.9
40	30.8±1.5	23.7±0.2	21.3±0.1	20.3±0.4	15.7±0.2	12.3±0.2
41	31.6±1.6	24.3±0.2	20.8±0.4	16.4±0.3	14.5±0.5	11±0.5
42	25.7±1	19.5±0.1	17.2±0.5	15.2±0.2	12.6±0.4	11.3±0.9
43	16.5±0.3	12.4±0.8	10.9±0.4	8.4±0.2	5±0.2	
44	23.4±0.8	19±0.5	16.9±0.3	14.8±0.1	11.5±0.4	7±0.2
45	22.5±0.7	16.2±0.3	13.2±0.2	9.4±0.1	7±0.1	4±0.2
46	30.4±1.5	23.9±0.3	20.1±0.4	16.9±0.3	10.6±0.3	0±0
47	39.3±2.8	30.3±0.2	25.6±0.2	20.1±0.2	17.7±0.2	11.4±0
48	50.2±5	40.7±0.5	38.7±0.2	38.7±0.2	35.7±0.2	33.7±0.5
49	40.2±2.9	31.7±0.3	24.5±0.4	21.7±0.1	18.9±1	12.9±0.5

50	30.6±1.5	21.7±0.2	19.6±0.2	14.4±1	8.9±0.3	3.4±0.2
51	28.7±1.3	22.1±0.2	19.3±0.5	14.9±0.3	12.6±0.9	10.6±0.6
52	38.7±2.7	27.5±0.2	26.7±0.1	22.9±0.2	17±0.9	14.7±0.2
53						
54	29.6±1.4	22.8±0.2	18.7±0.1	16.3±0.2	12.7±0.4	10.4±0.5
55	22.9±0.7	17.8±0.2	15.3±0.5	12.6±0.2	7.3±0.5	
56	39.3±2.8	27.9±0.2	24.6±0.8	21.5±0.2	18.1±0.5	13.8±0.5
57	86.3±17	66.4±0.2	57.8±0.5	45.7±0.6	32.8±0.2	27.6±0.5
58	36.6±2.4	28.6±0.2	23.8±0.2	20.5±0.3	15.8±0.4	10±0.6
59	28.9±1.3	19.7±0.9	18.7±0.2	16.5±0.5	12.6±0.6	4.5±1
60	42±3.3	27.2±0.2	24.4±1	18.1±0.4	9.8±0.2	7.1±0.1
61	28.2±1.2	23.1±0.6	18.6±0.3	16±0.5	13±0.4	8±0.9
62	31.2±1.6	24.3±0.2	22.8±0.4	20.9±0.5	17.1±0.2	11.2±0.2
63	46±4.1	34.5±0.8	30.4±0.4	26.7±1	21±0.3	17±0.6
64	36.9±2.4	28.8±0.2	24.9±0.7	19.5±0.6	16.2±0.9	6.7±0.7
65	27±1.1	21±0.2	19.1±0.2	17.5±0.2	13.8±0.2	11.9±0.9
66	25.3±0.9	19.5±0.2	16.4±0.2	13.7±0.1	10.6±0.1	3±0.5
67	23.2±0.7	16.9±0.4	15.5±0.5	12.8±0.2	8.6±0.6	4.2±0
68	30.3±1.5	23.6±0.2	18.5±0.4	15.7±0.3	11.5±0.2	3.7±0.4

69	32.1±1.7	23.4±0.4	21.5±0.5	18±0.3	13.8±0.4	11.9±0.6
70	39.8±2.9	26.7±0.5	21.5±0.1	12.7±0.5	10.3±0.4	4.4±0.2
71	32.7±1.8	25.5±0.2	23.2±0.2	23.2±0.2	20.3±0.7	17.7±0.1
72	39.5±2.8	31.2±0.3	24.9±0.1	22.5±0.6	18.2±0.5	15±0.2
73	38.92±2.7	31.9±0.6	29.6±0.1	24.5±0.1	19.1±0.4	15.6±0.2
74	41.14±3.1	32.5±0.3	31.7±0.2	27.6±0.5	22.2±0.1	17.7±0.4
75	29.52±1.4	23.9±0.5	20.7±0.2	19.8±0.5	17.4±0.2	14.8±0.9
76	66.84±0.9	56.9±0.1	55.3±0.8	54.7±0.6	54±0.5	51.7±0.2

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