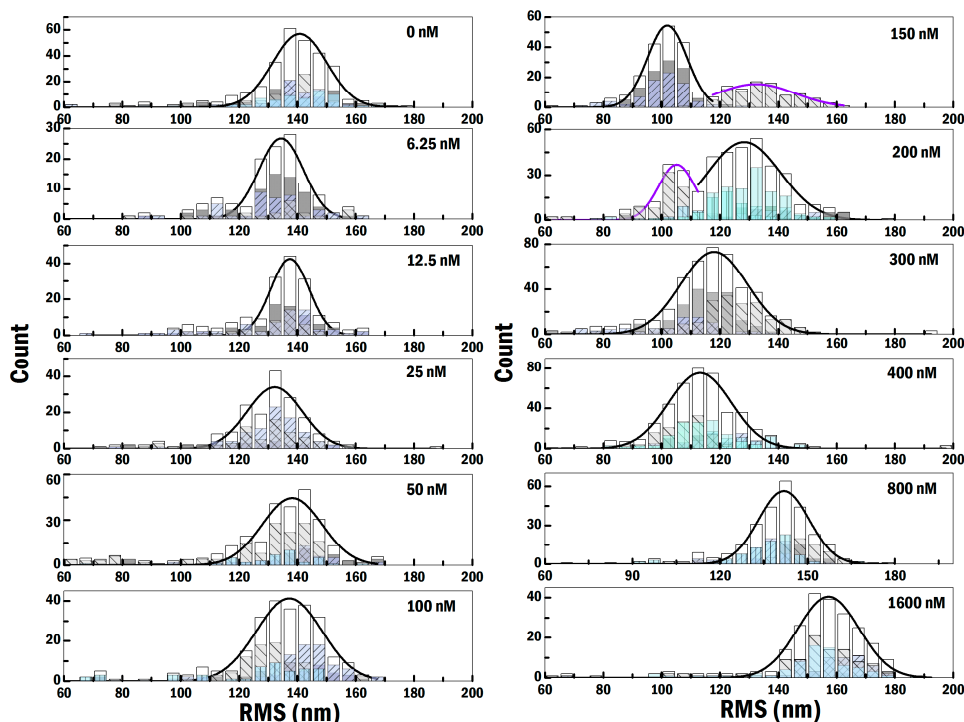
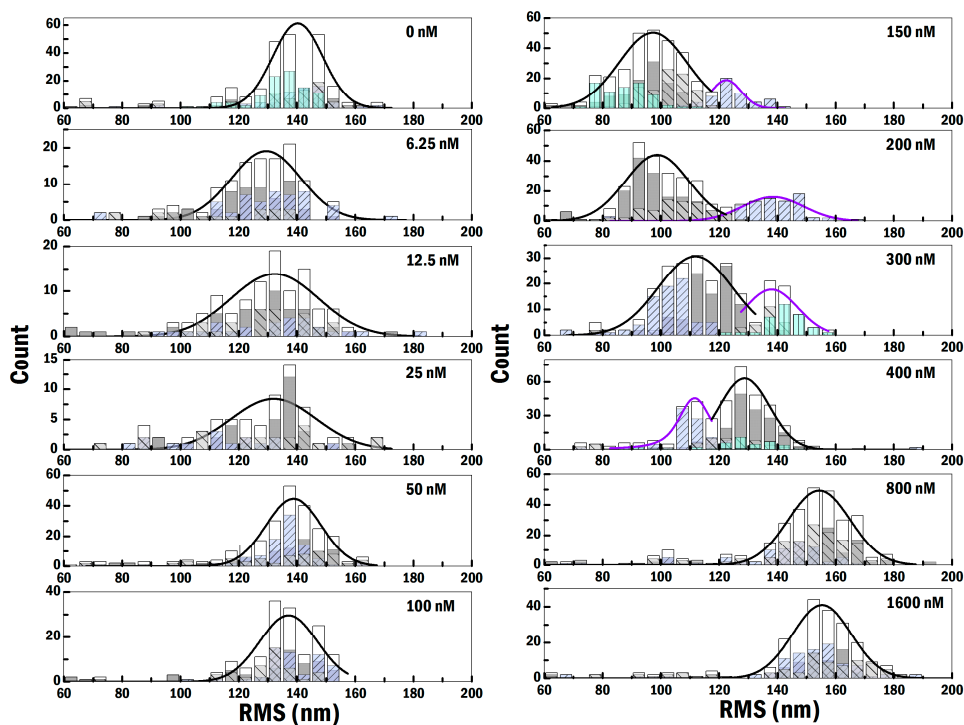


## Supplementary information

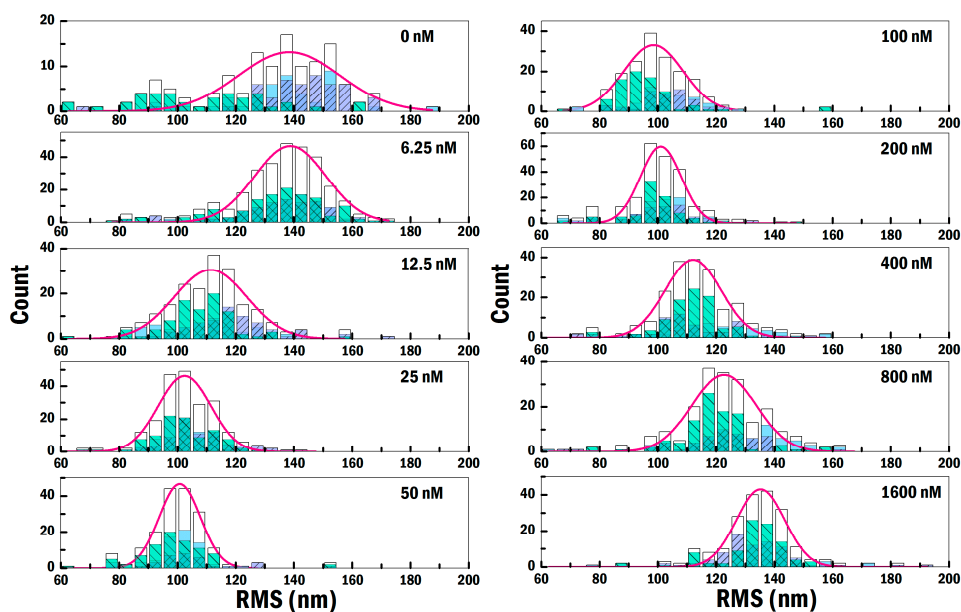
SI figure 1 to 5 include multiple independent experiments which are labelled with different colours and patterns.



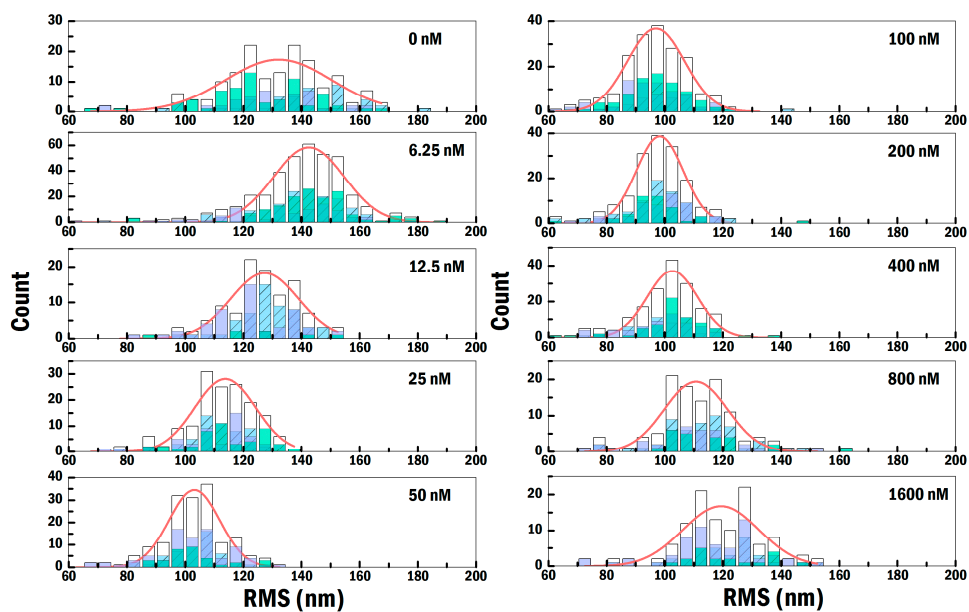
SI Figure 1A. Distribution of RMS values obtained at different HU concentration in the absence of MgCl<sub>2</sub>.



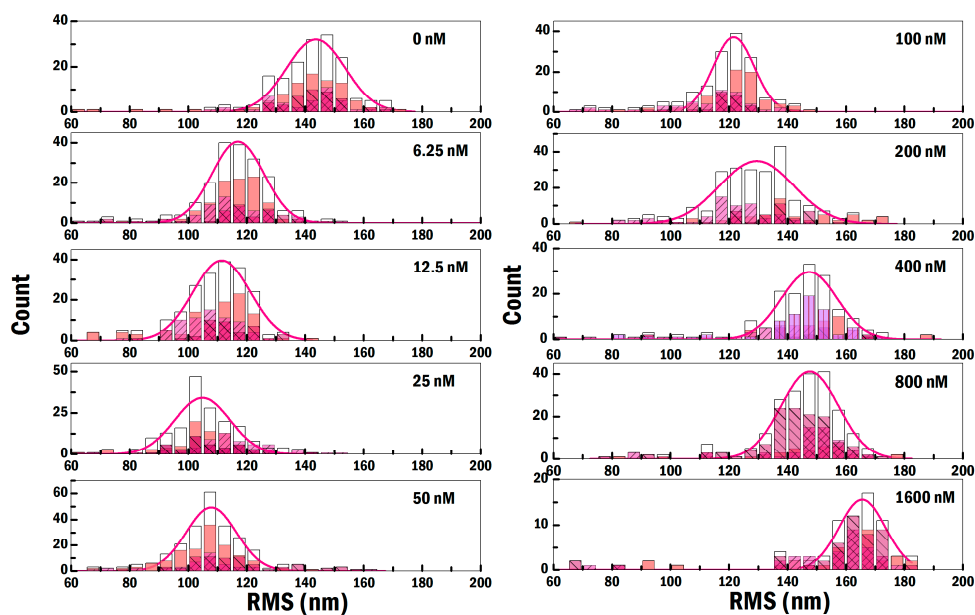
SI Figure 1B. Distribution of RMS values obtained at different HU concentration in the presence of MgCl<sub>2</sub>.



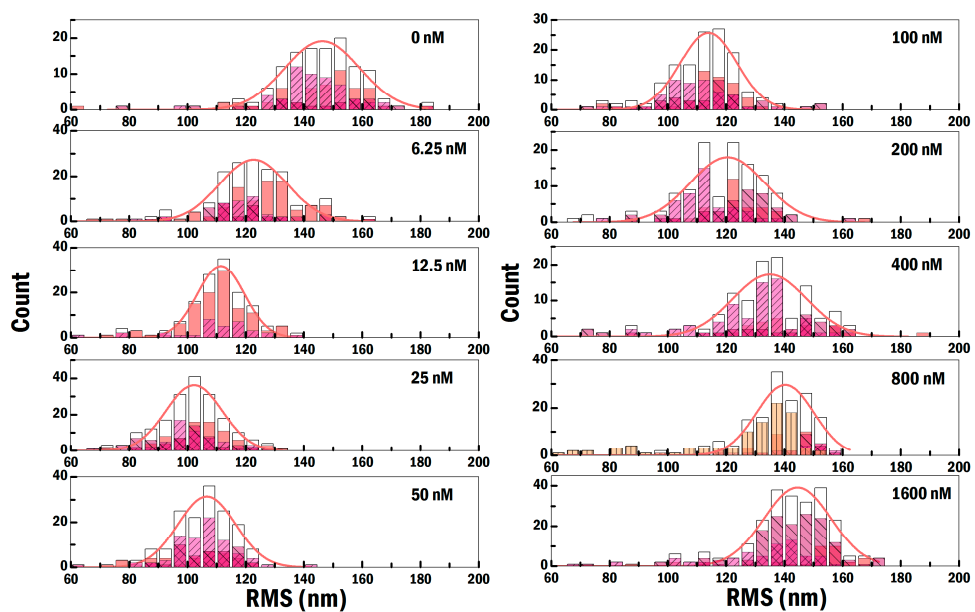
**SI Figure 2A. Distribution of RMS values obtained at different HU concentration in the presence of 0.5 % (w/v) BGB in the absence of MgCl<sub>2</sub>.**



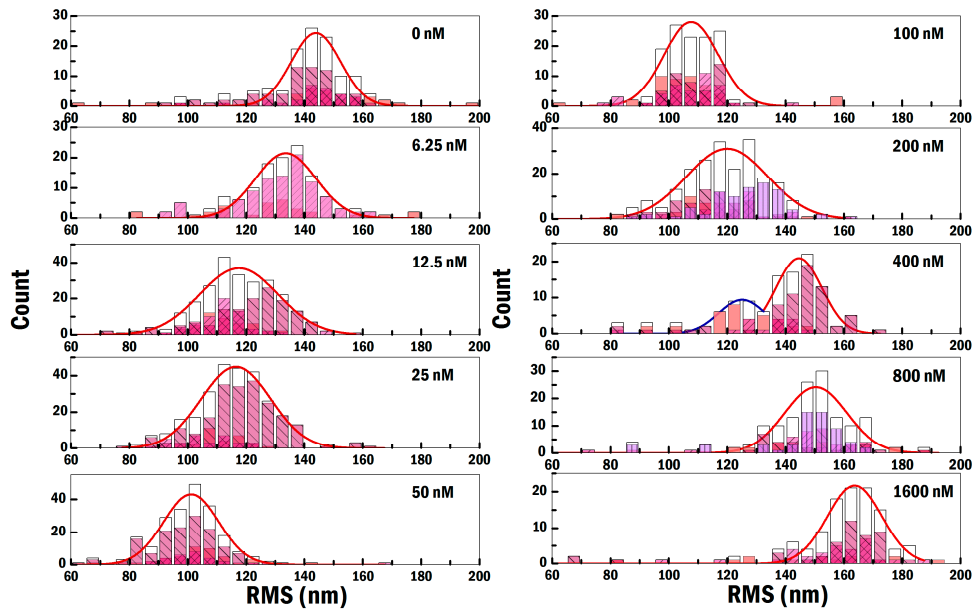
**SI Figure 2B. Distribution of RMS values obtained at different HU concentration in the presence of 1 % (w/v) BGB in the absence of MgCl<sub>2</sub>.**



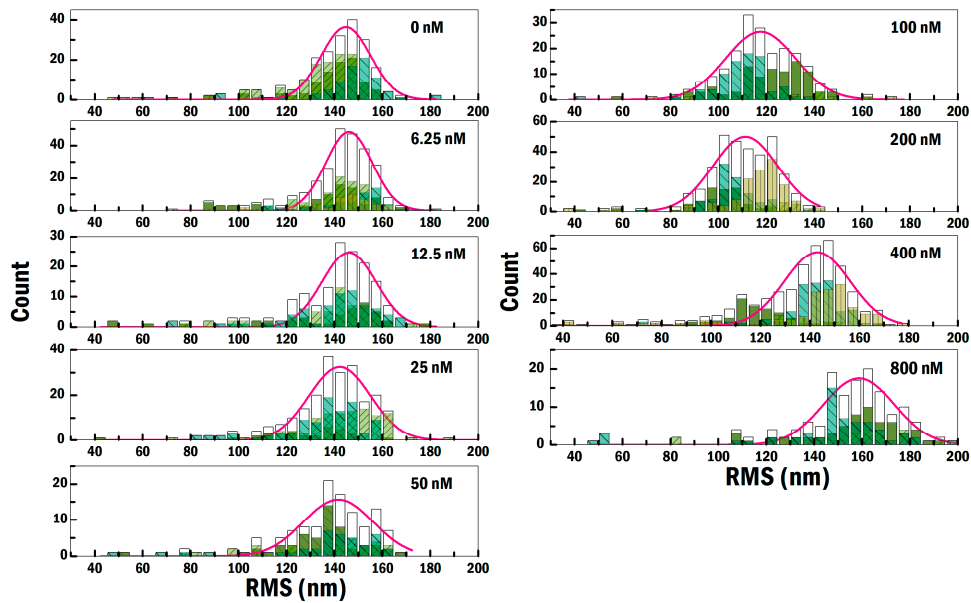
**SI Figure 3A. Distribution of RMS values obtained at different HU concentration in the presence of 1.25 % (w/v) BSA in the absence of  $MgCl_2$ .**



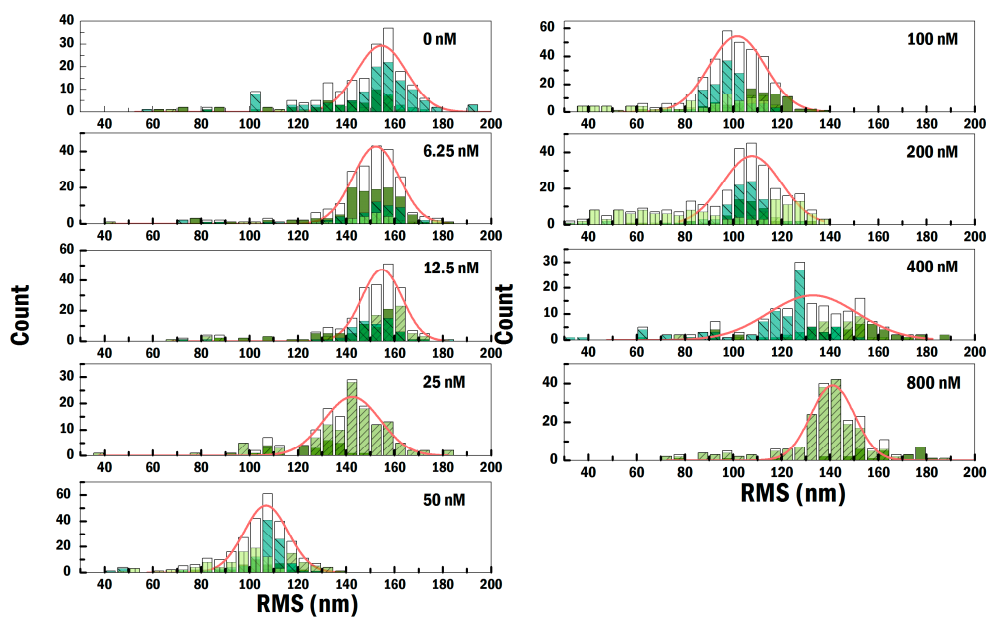
**SI Figure 3B. Distribution of RMS values obtained at different HU concentration in the presence of 5 % (w/v) BSA in the absence of  $MgCl_2$ .**



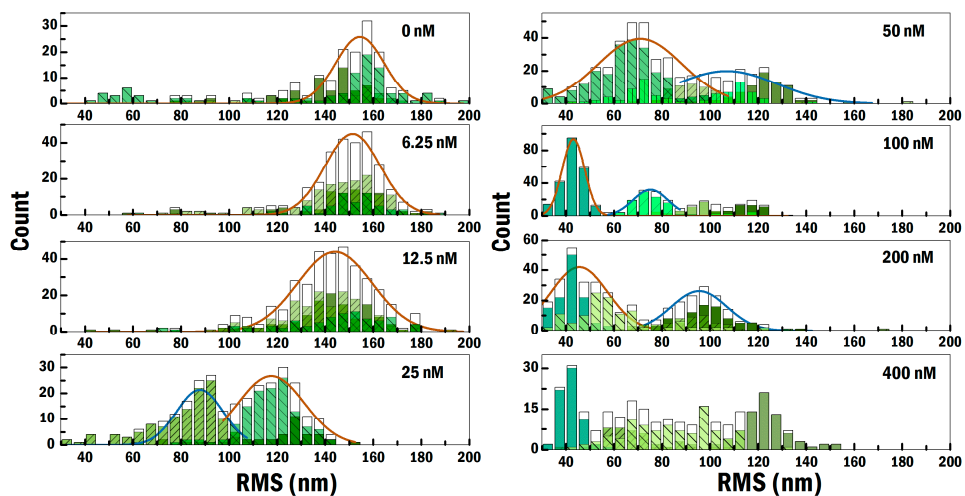
SI Figure 3C. Distribution of RMS values obtained at different HU concentration in the presence of 10 % (w/v) BSA in the absence of  $MgCl_2$ .



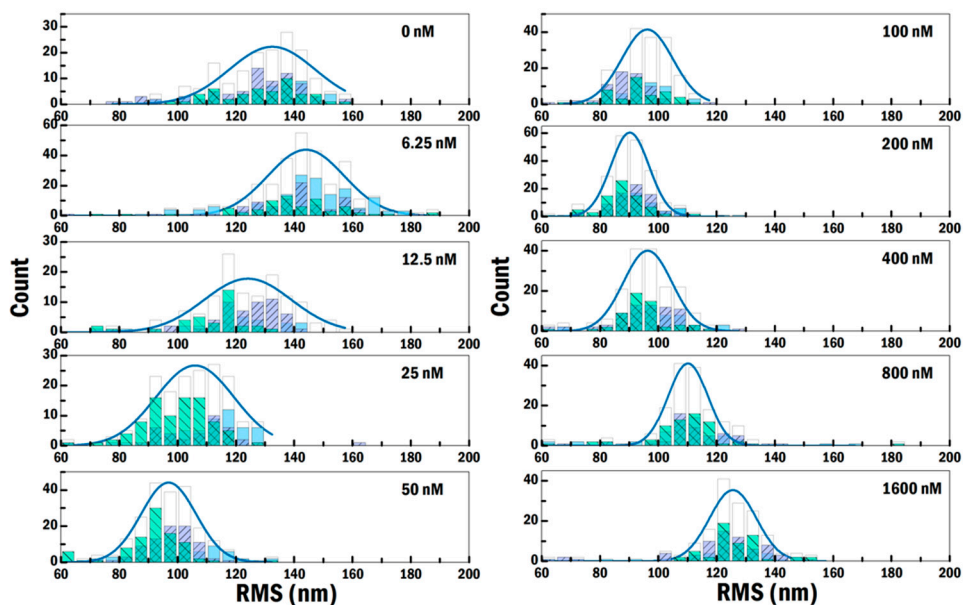
SI Figure 4A. Distribution of RMS values obtained at different HU concentration in the presence of 3 % (w/v) PEG8000 in the absence of  $MgCl_2$ .



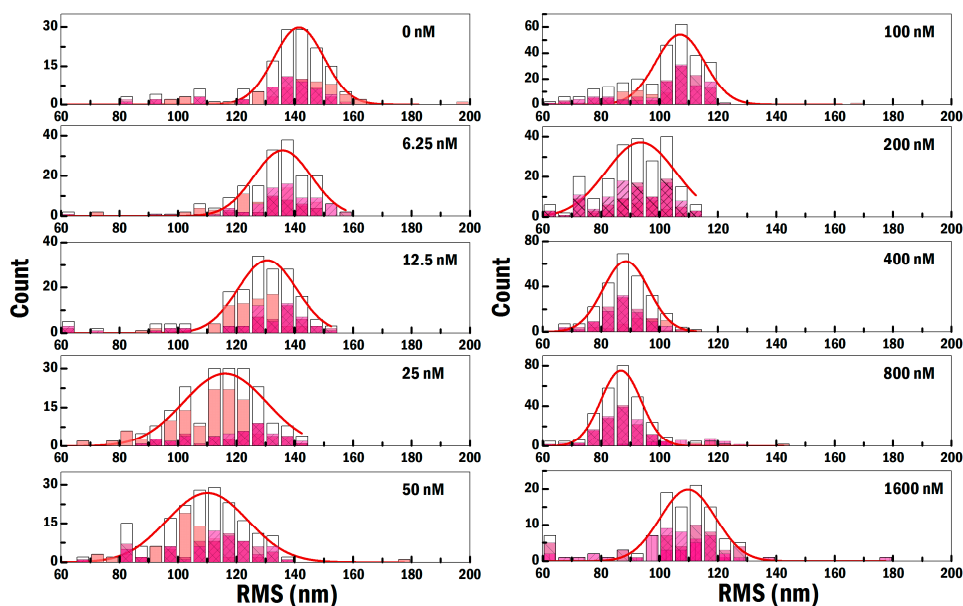
SI Figure 4B. Distribution of RMS values obtained at different HU concentration in the presence of 9 % (w/v) PEG8000 in the absence of  $MgCl_2$ .



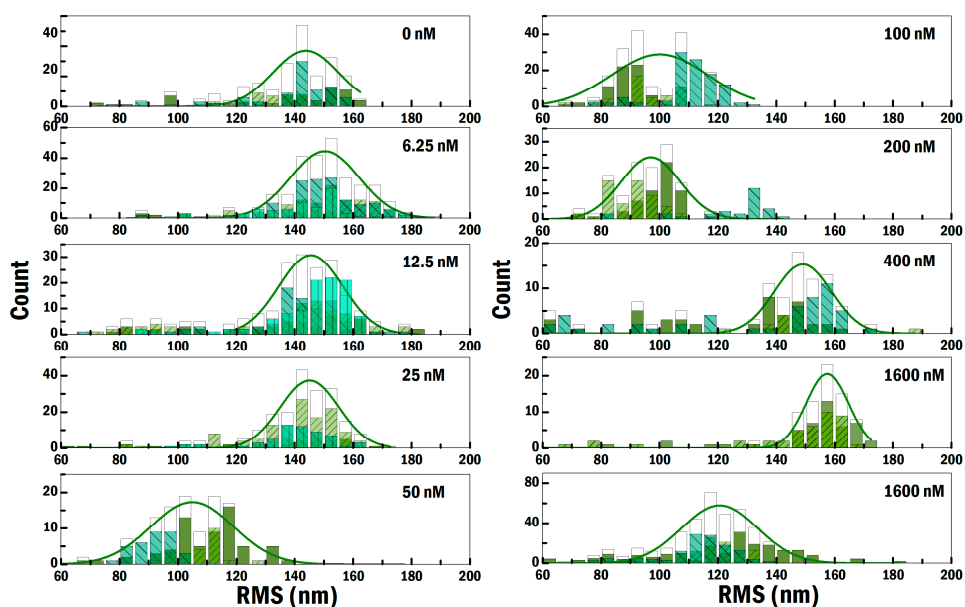
SI Figure 4C. Distribution of RMS values obtained at different HU concentration in the presence of 15 % (w/v) PEG8000 in the absence of  $MgCl_2$ .



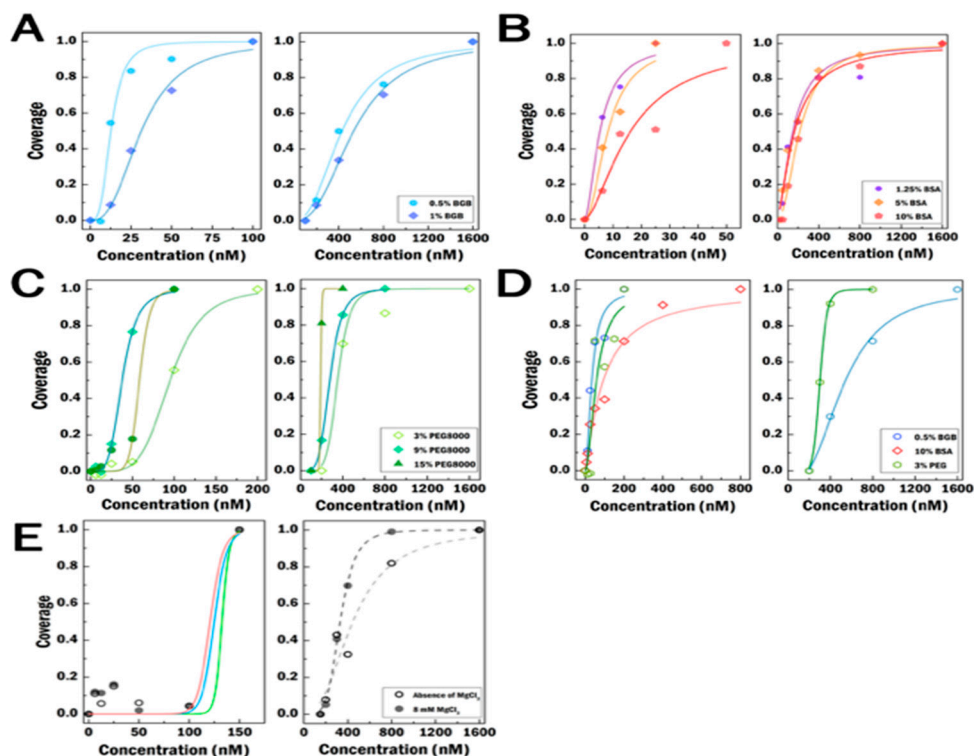
SI Figure 5A. Distribution of RMS values obtained at different HU concentration in the presence of 0.5 % (w/v) BGB in the presence of MgCl<sub>2</sub>.



SI Figure 5B. Distribution of RMS values obtained at different HU concentration in the presence of 10 % (w/v) BSA in the presence of MgCl<sub>2</sub>.



SI Figure 5C. Distribution of RMS values obtained at different HU concentration in the presence of 3 % (w/v) PEG8000 in the presence of  $MgCl_2$ .



SI Figure 6. The fractional coverage as a function of HU concentration fitted by Hill function. A, B, C are the fittings of two HU binding modes in the absence of  $MgCl_2$  and with BSA, BGB, and PEG8000, respectively. D are the fittings of two HU binding modes in the presence of  $MgCl_2$  and with BSA, BGB, and PEG8000, respectively. E is in crowder-free condition in the presence (empty circle) or presence (solid circle) of  $MgCl_2$ . In the left plot of E shows theoretical curves are shown with cooperativity value of  $19 \pm 2$  (red),  $20 \pm 2$  (blue), and  $38 \pm 7$  (green). For each plot: left) DNA binding behavior in the compaction regime; right) DNA binding behavior in the extension regime.

**SI Table 1. The RMS values of HU-DNA in presence and absence of MgCl<sub>2</sub>.**

HU Conc. (nM)	Without MgCl <sub>2</sub> *	N	With 8 mM MgCl <sub>2</sub> **	N
0	140.8 ± 0.4	300	140.3 ± 0.6	302
6.25	134.5 ± 0.7	128	129 ± 1	72
12.5	137.5 ± 0.6	180	132 ± 1	116
25	132.3 ± 0.7	180	129 ± 1	126
50	137.2 ± 0.6	224	138.9 ± 0.7	245
100	138.3 ± 0.4	306	137.1 ± 1	158
150	102.4 ± 0.7	198	97.3 ± 0.8	222
	132 ± 1	198	122 ± 1	42
200	128.3 ± 0.8	261	99 ± 1	244
	105.2 ± 0.6	172	139 ± 1	88
300	118.0 ± 0.5	390	111.9 ± 0.6	190
			138 ± 3	58
400	113.4 ± 0.7	393	125 ± 1	265
			111.6 ± 0.8	34
800	141.9 ± 0.4	195	154.4 ± 0.7	305
1600	157.2 ± 0.4	161	155.3 ± 0.4	235

\*Without MgCl<sub>2</sub>: 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM EDTA

\*\*Without 8 mM MgCl<sub>2</sub>: 10 mM Tris (pH 7.5), 50 mM NaCl, 8 mM MgCl<sub>2</sub>

**SI Table 2. The RMS values of HU-DNA in presence of BGB in buffer containing and absence of Mg<sup>2+</sup>.**

HU Conc. (nM)	0.5 % BGB without MgCl <sub>2</sub>	N	1 % BGB without MgCl <sub>2</sub>	N	0.5 % BGB without MgCl <sub>2</sub>	N
0	138 ± 2	134	132 ± 2	162	133 ± 2	171
6.25	138.8 ± 0.7	313	143 ± 0.6	385	144 ± 1	312
12.5	111.4 ± 0.9	201	127 ± 1	111	124 ± 2	118
25	102.5 ± 0.6	225	113.8 ± 0.9	153	106 ± 1	176
50	100.8 ± 0.4	183	103.2 ± 0.9	164	96.9 ± 0.6	227
100	98.5 ± 0.7	173	97 ± 0.4	191	96.2 ± 0.8	170
200	101 ± 0.6	246	98.3 ± 0.7	172	90.3 ± 0.4	22
400	112.1 ± 0.6	211	102.6 ± 0.05	175	96.4 ± 0.6	184
800	123 ± 1	211	111 ± 1	114	110.2 ± 0.5	171
1600	135.3 ± 0.4	205	119 ± 2	113	125.6 ± 0.6	162



**SI Table 3. The RMS values of HU-DNA in presence of BSA in buffer containing and absence of Mg<sup>2+</sup>.**

<b>HU Conc. (nM)</b>	<b>1.25 % BSA without MgCl<sub>2</sub></b>	<b>N</b>	<b>5 % BSA without MgCl<sub>2</sub></b>	<b>N</b>	<b>10 % BSA without MgCl<sub>2</sub></b>	<b>N</b>	<b>10 % BSA without MgCl<sub>2</sub></b>	<b>N</b>
<b>0</b>	143.7 ± 0.5	17 6	146.3 ± 0.7	12 8	144 ± 0.6	12 7	141.5 ± 0.5	15 5
<b>6.25</b>	117.0 ± 0.3	19 8	125 ± 1	17 3	133.6 ± 0.8	24 4	135.9 ± 0.8	17 9
<b>12.5</b>	111.5 ± 0.6	20 7	101.8 ± 0.5	44 1	117.6 ± 0.7	24 9	130.6 ± 0.7	18 8
<b>25</b>	105 ± 1	18 3	102.3 ± 0.5	19 0	116.5 ± 0.6	27 7	116 ± 2	20 5
<b>50</b>	107.9 ± 0.6	24 7	106.7 ± 0.7	16 2	101.2 ± 0.6	22 4	110 ± 1	19 4
<b>100</b>	121.6 ± 0.4	17 0	114 ± 0.7	14 0	107.7 ± 0.9	13 4	107.1 ± 0.9	27 5
<b>200</b>	130 ± 1	24 1	121 ± 2	11 9	120 ± 0.9	22 2	94 ± 1	22 5
<b>400</b>	147.5 ± 0.6	16 5	135 ± 1	12 1	142 ± 1	11 9	88.5 ± 0.4	25 9
<b>800</b>	147.6 ± 0.5	22 1	140.3 ± 0.8	16 8	150.4 ± 0.9	14 5	86.8 ± 0.3	28 4
<b>1600</b>	165.6 ± 0.6	11 1	144.6 ± 0.7	24 7	163.7 ± 0.6	12 3	112 ± 2	21 2

**SI Table 4. The RMS values of HU-DNA in presence of PEG8000 in buffer containing and absence of MgCl<sub>2</sub>.**

<b>HU Conc. (nM)</b>	<b>3 % PEG8000 without MgCl<sub>2</sub></b>	<b>N</b>	<b>9 % PEG8000 without MgCl<sub>2</sub></b>	<b>N</b>	<b>15 % PEG8000 without MgCl<sub>2</sub></b>	<b>N</b>	<b>3 % PEG8000 without MgCl<sub>2</sub></b>	<b>N</b>
<b>0</b>	144.8 ± 0.6	21 4	155 ± 1	19 4	154 ± 1	18 7	144 ± 2	19 2
<b>6.25</b>	146.1 ± 0.6	27 1	152.3 ± 0.5	23 1	151.4 ± 0.5	28 6	150.3 ± 0.9	29 2
<b>12.5</b>	146.1 ± 0.9	16 4	154.8 ± 0.6	20 0	144.1 ± 0.7	36 3	145.6 ± 0.9	
<b>25</b>	142.3 ± 0.7	22 7	143 ± 1	15 6	118 ± 1	14 7	145.1 ± 0.7	27 8
					88 ± 1	14 6		
<b>50</b>	142 ± 1	12 3	109.6 ± 0.6	27 3	107 ± 2	17 3	105 ± 1	12 5
					71 ± 2	27 5		
<b>100</b>	117.7 ± 0.9	19 9	102.2 ± 0.4	26 0	104 ± 3	21 0	90.2 ± 2	22 9
					43.1 ± 0.4	24 6		
<b>200</b>	104.3 ± 0.7	34 3	106.3 ± 0.4	29 9	97 ± 0.8	14 7	97 ± 2	14 3
					41 ± 1	25 1		
<b>400</b>	143 ± 1	44 5	133 ± 2	17 6	122 ± 0.7	---	149 ± 1	11 1
<b>800</b>	159 ± 1	14 4	141.2 ± 0.8	22 5	---	---	157.5 ± 0.4	91
<b>1600</b>	---	---	---	---	---	---	117.4 ± 0.5	43 8

**SI Table 5. Fitting values of Hill function.**

<b>Conditions</b>	<b>Compactio n regime</b>	<b>Extensio n regime</b>
<b>Without MgCl<sub>2</sub></b>	---	2.5 ± 0.6
<b>With 8 mM MgCl<sub>2</sub></b>	---	4.9 ± 0.4
<b>0.5 % BGB</b>	3.3 ± 0.9	2.4 ± 0.3
<b>1 % BGB</b>	2.6 ± 0.7	2.5 ± 0.3
<b>1.25 % BSA</b>	1.7 ± 0.5	1.4 ± 0.2
<b>5 % BSA</b>	2.0 ± 0.7	2.0 ± 0.2
<b>10 % BSA</b>	1.6 ± 0.6	1.6 ± 0.2
<b>3 % PEG8000</b>	5.0 ± 0.8	6 ± 5
<b>9 % PEG8000</b>	4.3 ± 0.2	4.9 ± 0.1
<b>15 % PEG8000</b>	10 ± 5	35 ± 0
<b>0.5 % BGB with MgCl<sub>2</sub></b>	1.8 ± 0.5	2.7 ± 0.7
<b>10 % BSA with MgCl<sub>2</sub></b>	1.2 ± 0.2	---
<b>3 % PEG8000 with MgCl<sub>2</sub></b>	1.8 ± 0.7	2.7 ± 0.7