Supplementary Information for:

Protein-mediated looping of DNA under slight tension requires supercoiling

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Table S1. The following combinations of plasmid templates and primers were used to produce the DNA segments used in this work. The sequences of the primers are given below and the sequences of the plasmids are described in previously published reports.

construct	Os-400-O1 (TPM)	O2-400-O1 (TPM)	O2-400-O1 (MT)	bio-, dig-tails
template	pYY_I1_400	pO2O1	pO2O1	pBluescriptKS(+)
forward primer	B-S/JBOIDO1_400/2345	D-S/pO1O2_401/1588	S/pO1O2_401/929_XmaI	S/pUC19/2412
reverse primer	D-A/JBOIDO1_400/3254	B-A/pO1O2_401/2418	A/pO1O2_401/3043_ApaI	A/pUC19/1435
restriction enzymes	-	-	XmaI, ApaI	XmaI, ApaI

primer name	primer sequence (5' to 3')
B-S/JBOIDO1_400/2345	bio-tgggaaggagaagataagatgg
D-A/JBOIDO1_400/3254	dig-cgttagggtcaatgcgggtc
B-S/pIT_Loop3/375-24	bio-gctagcttcttcgtctgtttctac
D-A/pIT_loop3/1769	dig-aggcaaagcgccattcg
D-S/pO1O2_401/1588	dig-tgctcgcttcgctacttg
B-A/pO1O2_401/2418	bio-tgactgggttgaaggctc
S/pO1O2_401/929_XmaI	tgcccgggacccggaaagacatgc
A/pO1O2_401/3043_ApaI	ctgggcccggtgaatccgttagcga
S/pUC19/2412	tgggtgagcaaaaacaggaaggca
A/pUC19/1435	gcgtaatctgctgcttgcaa

Table S2. For the LacI and HU titrations the motions of 32 to 137 beads were recorded for each condition. Most exhibited symmetrical motion, indicative of a single tethering molecule. In addition, approximately 74% of them also displayed the expected amplitude of excursions without added LacI or HU.

overall average of usable beads	73.9

LacI titrations

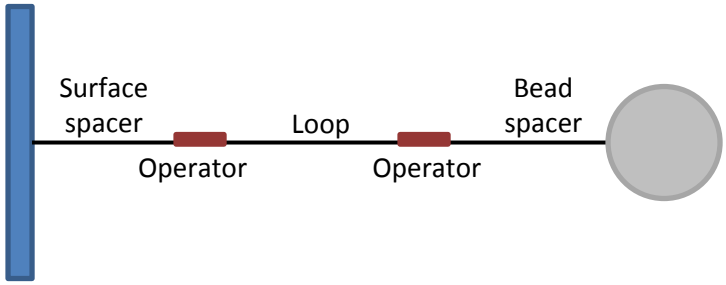
	Os-400-O1				O1-400-O2		
[LacI] (nM)	Tracked	Selected	percentage (%)	[LacI] (nM)	Tracked	Selected	percentage (%)
0	32	24	75	0	54	47	87
0.001	74	56	76	0.01	77	66	86
0.003	96	84	88	0.02	51	41	80
0.01	88	63	72	0.05	65	56	86
0.03	54	40	74	0.1	56	39	70
0.04	54	38	70	0.2	67	51	76
0.05	34	26	76	0.5	52	43	83
0.1	76	52	68	1	83	70	84
0.15	52	33	63	2.5	90	73	81
0.2	51	34	67	5	63	47	75
0.5	58	36	62	10	70	39	56
1	48	34	71	20	62	44	71
2	46	32	70				
5	46	32	70				
10	46	34	74				
20	33	25	76				

HU titrations

	Os-400-O1			O1-400-O2		
[HU] (nM)	Tracked	Selected	percentage (%)	Tracked	Selected	percentage (%)
0	90	60	67	90	63	70
4.4	75	64	85	65	48	74
22	94	67	71	131	93	71
44	79	68	86	78	53	68
66	107	77	72	139	105	76
88	76	46	61	85	65	76
110	123	89	72	125	93	74
132	137	93	68	164	135	82
264	98	67	68	136	103	76
528	108	69	64	150	108	72
1056	84	63	75	139	111	80

Table S3. Observed mean squared excursion as well as the apparent contour lengths and fractional extensions were as follows for ensembles of tethered beads exposed to the indicated concentrations of HU. After the analysis described in the material and methods, percentages of looping associated with these ensembles were calculated.

[HU] (nM)	$\langle\rho^2\rangle_{unloop}$ (nm^2)	Apparent contour length (nm)	fractional-extension	P_{loop} (%)
Os-400-O1				
0	36228.33	324.94	1.00	21.97
4.4	35250.00	315.24	0.97	25.05
22	33469.38	297.60	0.92	41.62
44	32169.72	284.71	0.88	34.51
66	30920.53	272.33	0.84	55.27
88	29719.85	260.43	0.80	56.01
110	28565.79	248.99	0.77	61.54
132	27456.55	238.00	0.73	68.18
264	24790.38	211.57	0.65	74.16
528	22433.90	188.21	0.58	79.07
1056	21000.61	174.01	0.54	78.46
O1-400-O2				
0	33469.38	297.60	1.00	23.30
4.4	32475.00	287.74	0.97	28.22
22	31169.72	274.80	0.92	33.46
44	29719.85	260.43	0.88	41.15
66	28565.79	248.99	0.84	55.37
88	27456.55	238.00	0.80	59.91
110	26519.85	228.71	0.77	63.57
132	25365.61	217.27	0.73	67.94
264	22523.93	189.11	0.64	75.50
528	20000.61	164.10	0.55	82.94
1056	19223.96	156.40	0.53	82.18



Surface spacer (bp)	20 bp Operator (type)	Loop (bp)	20 bp Operator (type)	Bead spacer (bp)	assay
256	Os	400	O1	253	TPM
194	O1	400	O2	237	TPM
843	O1	400	O2	854	MT

Figure S1: Schematic diagrams of the DNA constructs. The DNA templates had a central loop segment with flanking (spacer) sequences on either side attached to the surface or the bead through digoxigenin- or biotin-labeled segments respectively. The lengths of the segments and operator types are indicated in the table as well as the types of assays in which the DNA was used.

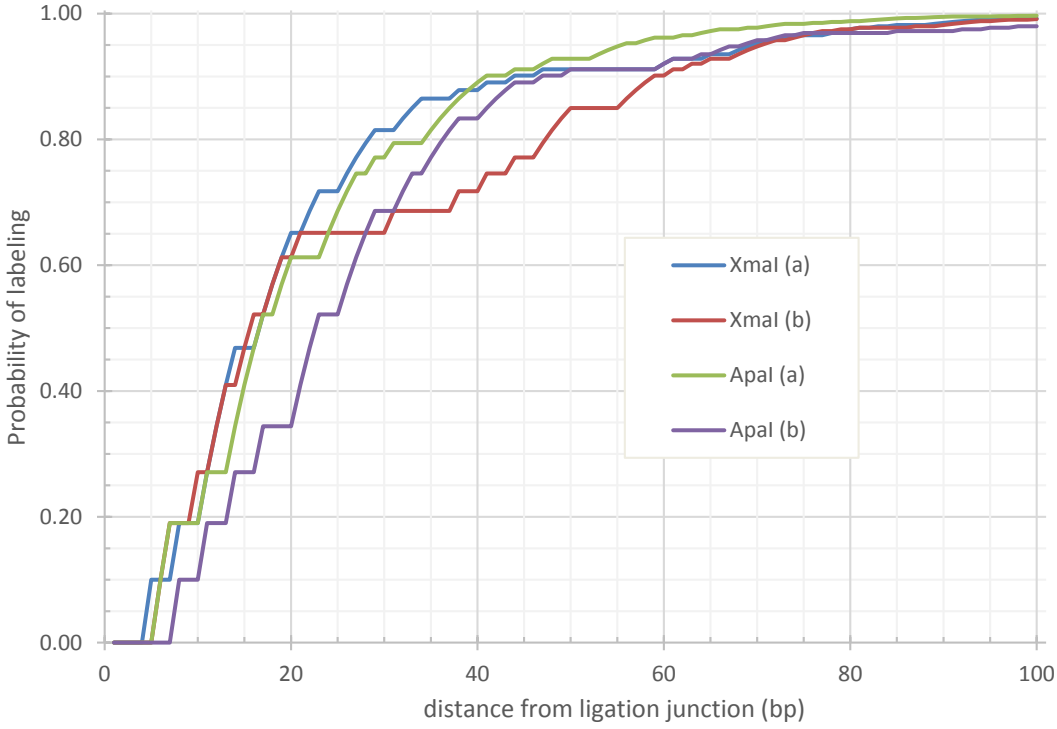


Figure S2: The probability of labeling along the anchorage fragment as a function of the distance from the ligation junction with the main fragment. The anchorage fragments are prepared by digesting with XmaI or ApaI approximately 2000 bp-long, biotin- or digoxigenin-labeled DNA amplicons centered on the multi-cloning site of pBluescriptKS+. Since the labels are attached to dUTP and the sequences are known, the probability of incorporating labeled nucleotides can be calculated as a function of the distance from the ligation junction (x) as the complement of the probability of not incorporating any label within that span. Since the fraction of labeled nucleotides was 0.1, the probability of labeling at each adenine nucleotide was 0.1. The probability that not even one label would have been incorporated within a segment *x* nucleotides long was $P_{unlabeled} = 0.9^{N_{A,x}}$, where $N_{A,x}$ is the number of adenines in the segment. Then the probability that at least one label will be incorporated in the segment is $P_{label} = 1 - 0.9^{N_{A,x}}$. There is about a 0.95 probability that at least one label will be incorporated within 70 bp from the junction. The variation of the effective tether length due to labeling will range from 14 to 127 bp or about 38 nm.

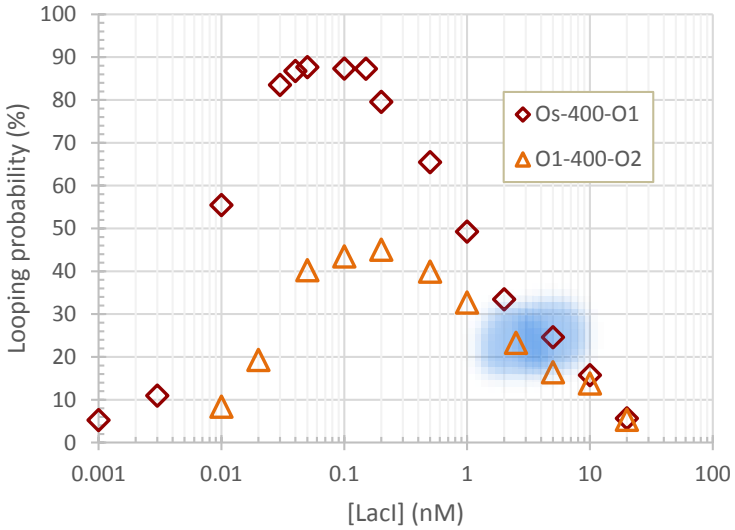
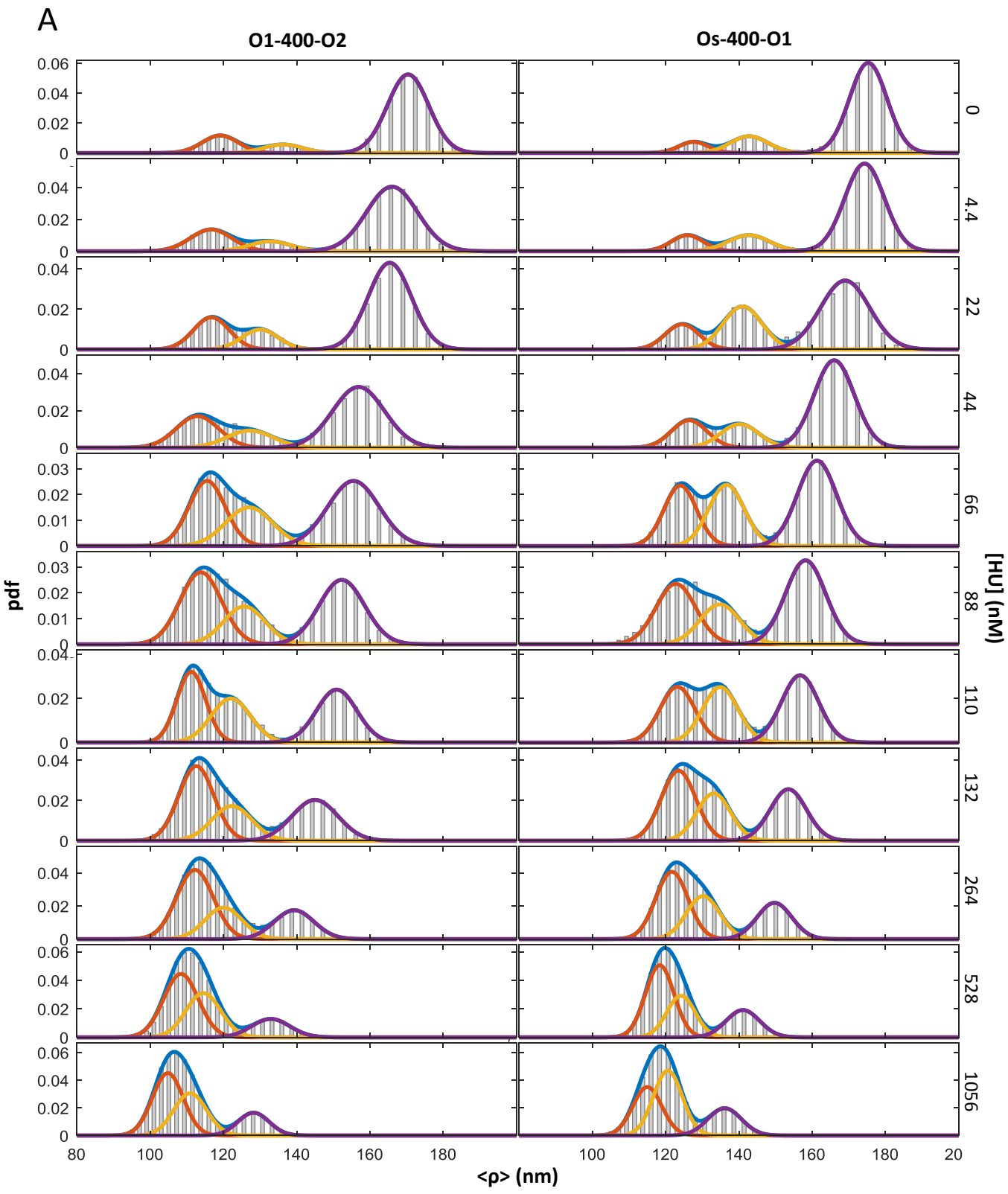


Figure S3: The looping probability was measured as a function of LacI concentration for 400 bp loops secured by either Os and O1 or O1 and O2 operators. Os-O1 looping probability peaked at 87% while O1-O2 only reached 45%. This is due to the separation between K_d values for Os and O1 compared to O1 and O2. LacI concentrations for which the measured looping percentage was near 25% (2.5 and 5 nM); cyan glow) were chosen for subsequent titrations of HU protein.


A
O1-400-O2
Os-400-O1
pdf
<ρ> (nm)
[HU] (nM)
0
4.4
22
44
66
88
110
132
264
528
1056

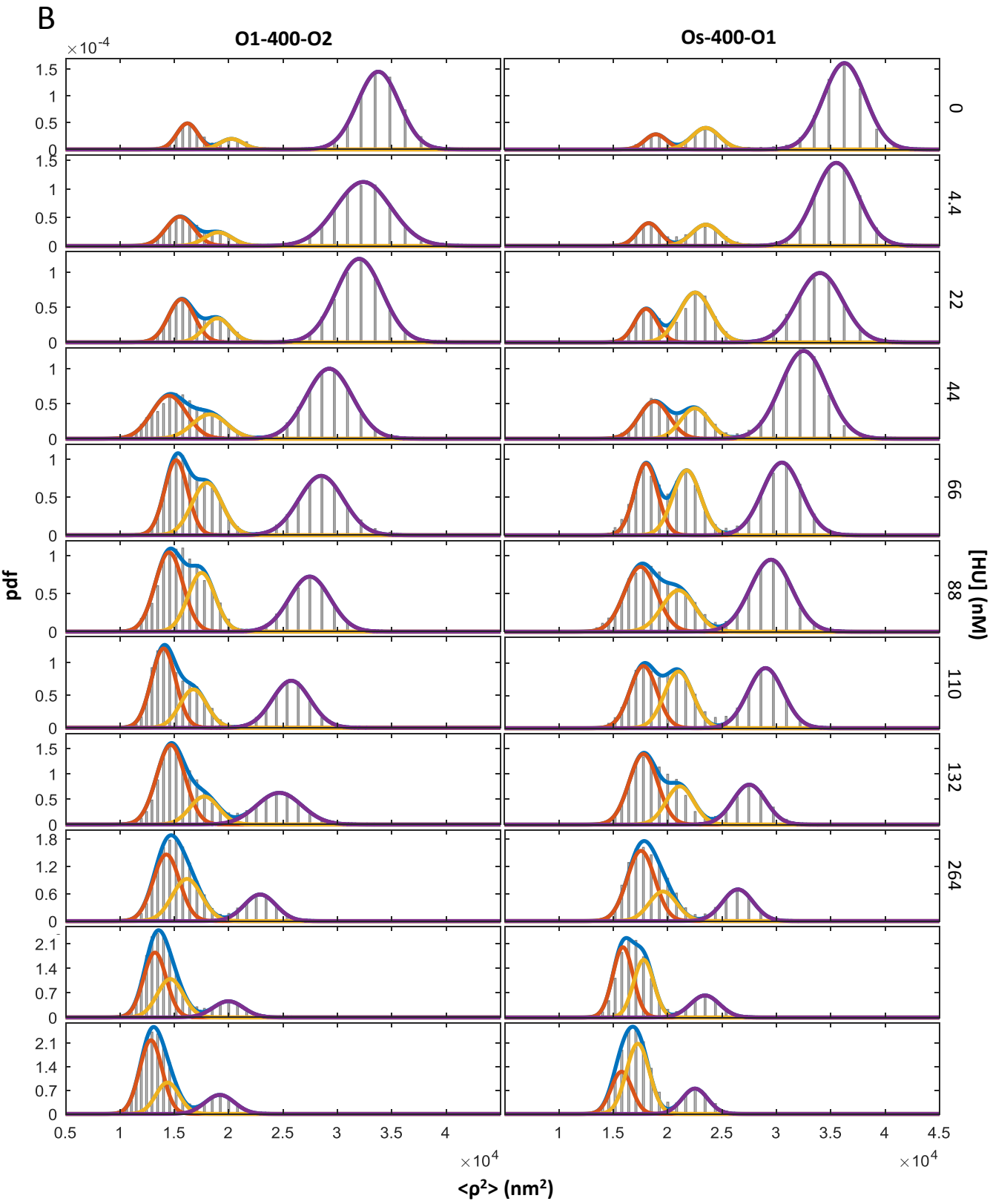

B
O1-400-O2
Os-400-O1
pdf
[HU] (nM)
0
4.4
22
44
66
88
110
132
264
<ρ²> (nm²)

Figure S4: Histograms of the measured excursion values, $<\rho>$ in (A) or $<\rho^2>$ in (B), for O1-400-O2 and Os-400-O1 DNA tethers in the presence of 5 or 2.5 nM LacI respectively and increasing (top to bottom) concentrations of HU protein. Histograms have been fitted with a sum of three Gaussian curves (blue) corresponding to two looped states (red and yellow) and one unlooped state (purple). The sum of the three components (blue) is visible for shorter excursions and superimposes with the purple for longer excursions. For both tether types, the excursion values for looped and unlooped states decreased as [HU] increased. Simultaneously, the fraction of time spent in looped states increased, as shown by the fraction of area corresponding to the looped state Gaussian components.

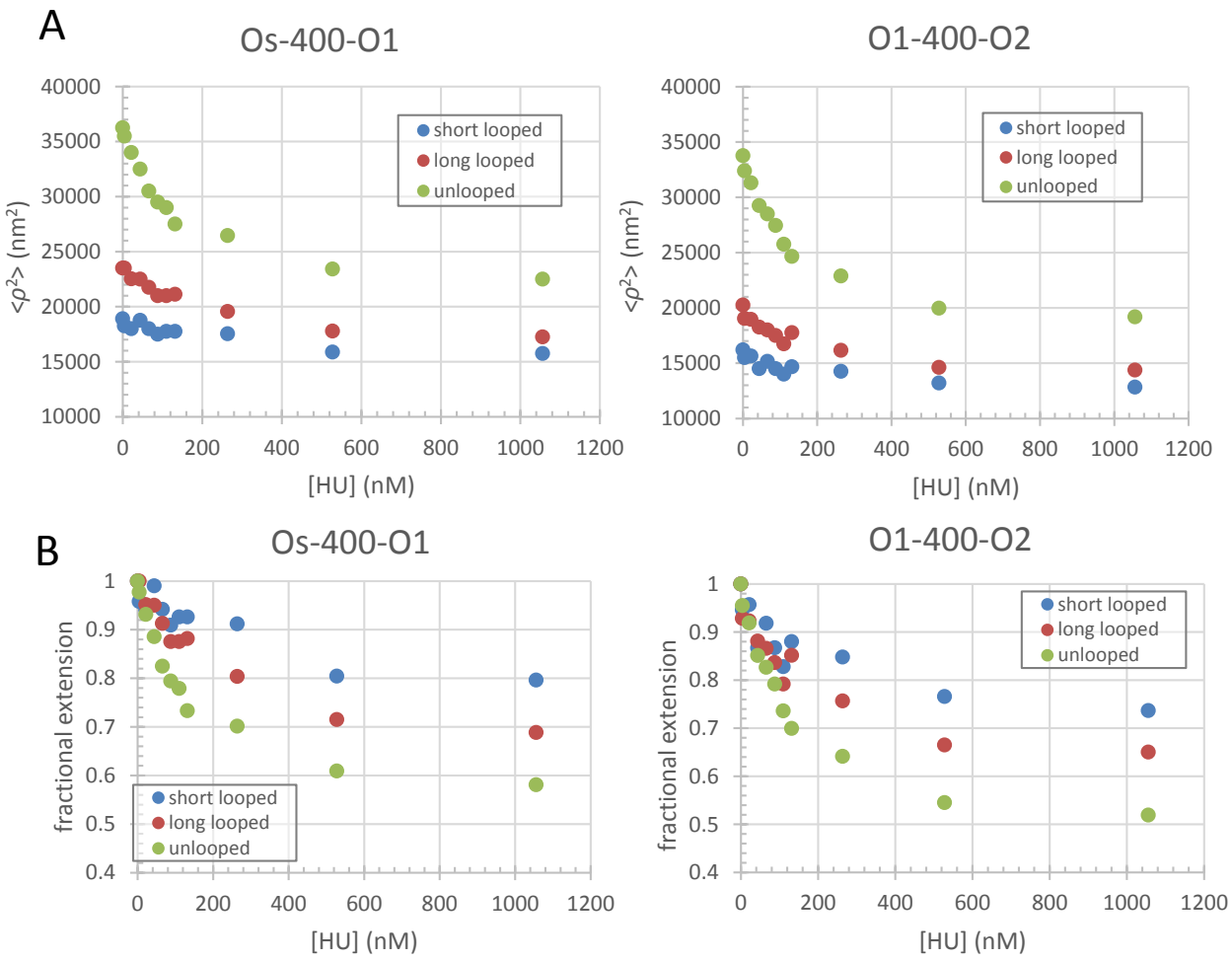


Figure S5. Increasing [HU] compacted DNA tethers. (A) The mean squared excursions of the two looped and the single unlooped conformations decreased as the HU concentration was increased, reaching a lower limit near 1 uM. (B) Plots of the extension as a function of HU concentration show that the unlooped tether exhibited a larger fractional decrease. This likely reflects the increased compaction due to bends in the center of the tether with respect to those near the ends.

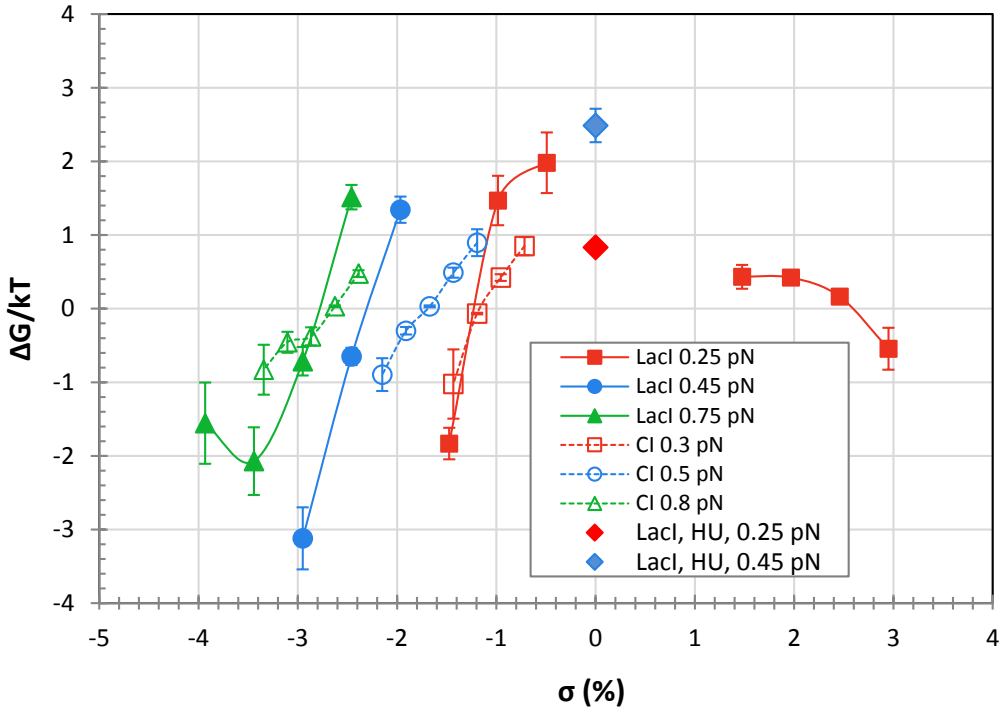


Figure S6: Free energy of looping ($\frac{\Delta G}{kT}$) for 400 bp, Lac- and 393 bp, Lambda-loops versus σ. Increasing negative supercoiling compensated for increased tension to induce spontaneous looping by both the lac (filled symbols) and lambda (hollow symbols) repressor proteins. Loop formation by the lac repressor appears to be more sensitive to supercoiling. Under low tension (0.25 pN, red symbols) saturating [HU] (diamonds) catalyzed a low level of looping but supercoiling (squares) more efficiently enhanced looping (ΔG ≤ 0). Under intermediate tension (0.45 pN, blue symbols), saturating HU (diamonds) produced very little looping (ΔG > 0), but negative supercoiling to greater extent effectively enhanced looping. Under high tension (0.75 pN, green), saturating HU could not induce looping (infinite ΔG) but negative supercoiling beyond levels used at lower tensions effectively enhanced supercoiling. Some error bars fall within symbols.

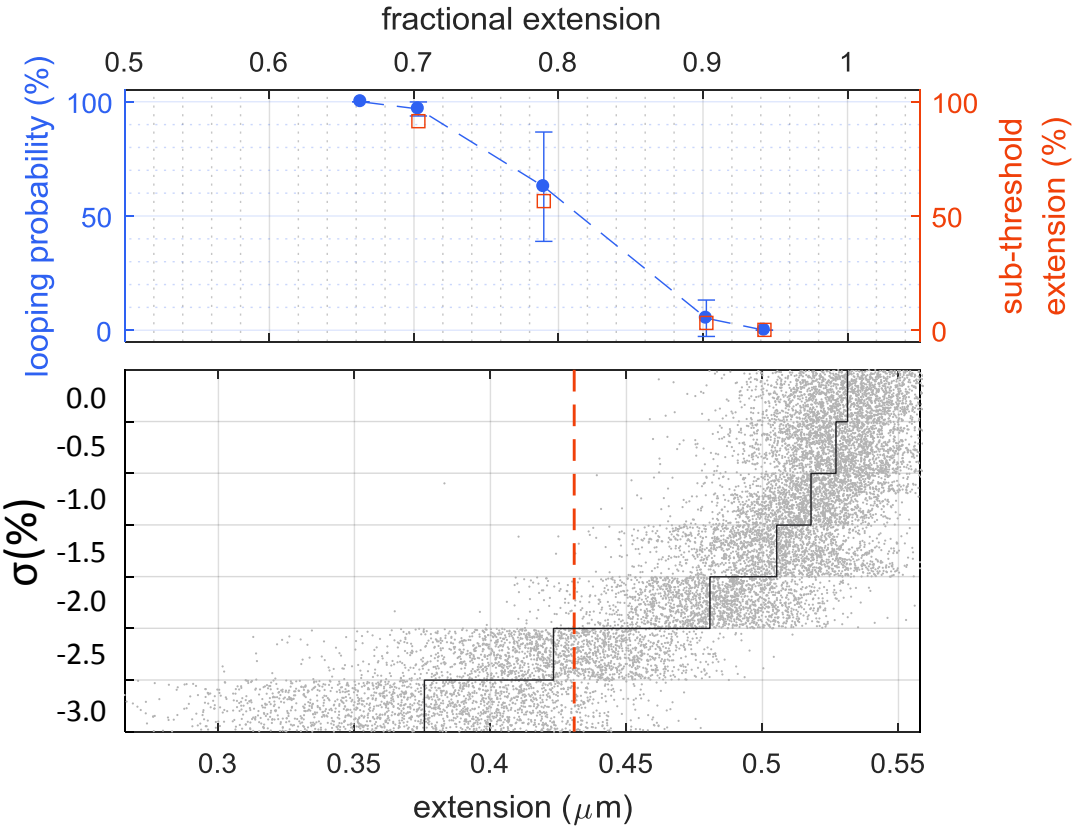


Figure S7: Supercoil-induced contraction of DNA tethers favors looping. (Lower) Negatively twisting the DNA reduced the extension of the DNA tether (black line is average at each level of twist) and drove progressively more instantaneous extension measurements (grey points) below a threshold level corresponding to a tether one loop segment shorter (red dashed line). (upper) The percentage of instantaneous extension measurements falling below the threshold (red squares) correlated with the rise in looping probability (blue circles) measured for the corresponding fractional extension of the DNA.