Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information

Probing the hierarchical dynamics of DNA-sperm nuclear transition protein complex through fuzzy interaction and mesoscale condensation

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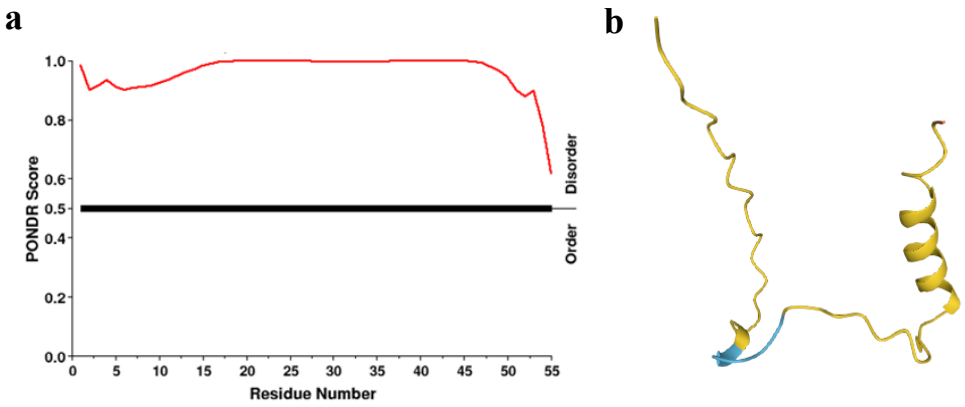


Figure S1: Structural prediction of TNP1. (a) Prediction of disorder regions via PONDR web server (http://www.pondr.com/pondr-tut2). (b) Structural prediction of TNP1 via AlphaFold 2 indicates overall intrinsic disorder. A small helix is predicted between residues 38 to 49, but the confidence is low.

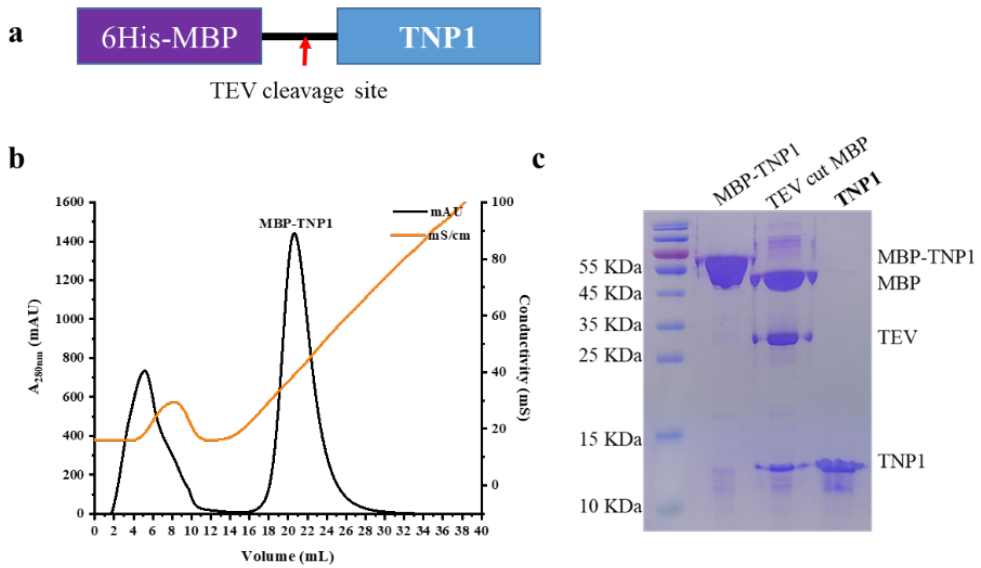


Figure S2: Expression and Purification TNP1. (a)Plasmid construct. (b) Anion-exchange chromatography with a HiTrap S column. (c) SDS-Page of MBP-TNP1,TEV cut MBP-tag and TNP1.

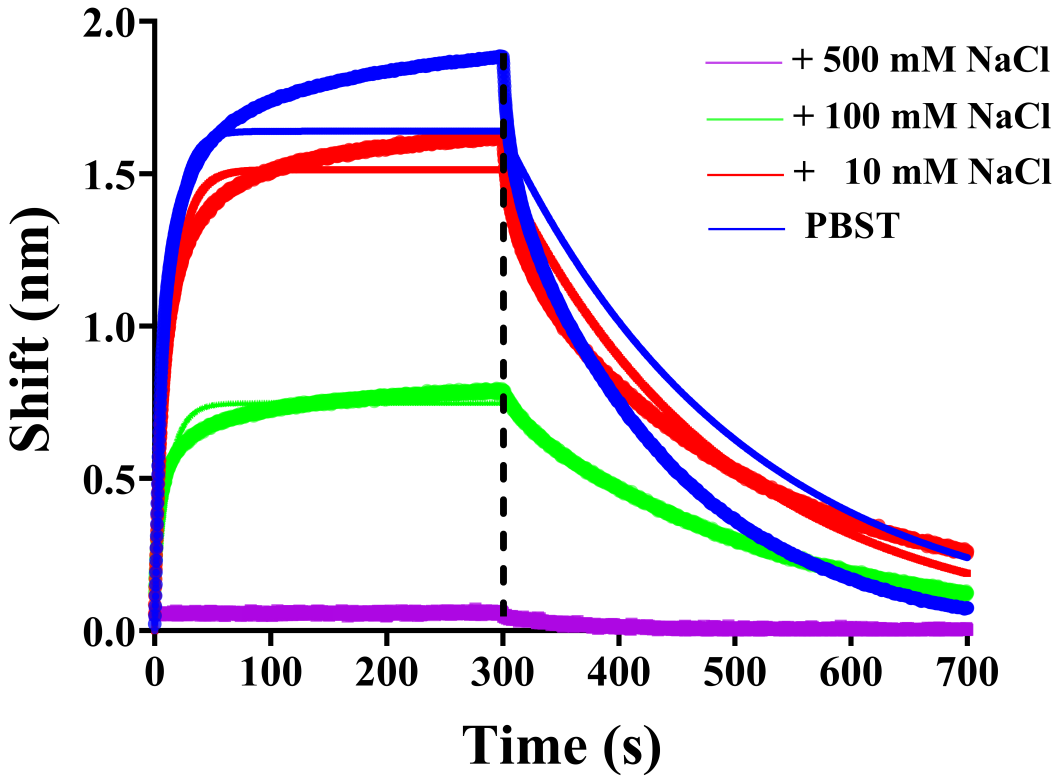


Figure S3: Influence of Salt Concentration on the Association of TNP1 (4 µM) with 177 bp DNA. PBS-T buffers were supplemented with additional NaCl to elevate the salt concentration by increments of 10 mM, 100 mM, and 500 mM, respectively.

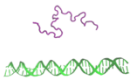
Table S1: List of computational systems

Conventional MD Simulation				
	1st TNP1	2nd TNP1	3rd TNP1	4th TNP1
Time	100 ns	100 ns	100 ns	100 ns
Repeat	3	3	3	3
Number of TNP1	1	2	3	4
Number of DNA	1	1	1	1
Number of water	~ 69500			
Number of ions	455	436	417	398

MetaDynamics & Conventional MD Simulation		
	MetaDynamics Simulation	Convential Simulation
Time	20 ns	30 ns
Repeat	20	
Number of TNP1	1	
Number of DNA	1	
Number of water	~ 70000	
Number of ions	455	

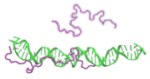
WCA potential based MesoMD Simulation					
	$R_{+/-}$ = 0.3	$R_{+/-}$ = 0.5	$R_{+/-}$ = 0.7	$R_{+/-}$ = 1.0	$R_{+/-}$ = 1.4
Time	100 ns				
Repeat	3				
Number of TNP1	30	60	90	120	180
Number of DNA	30				
Number of water	Implict Solvent				
Number of ions	2970	3540	4110	4680	5820

First TNP1



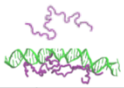
	Van der Wall Energy	Electrostatic energy	Polar solvation Energy	SASA Energy	Binding Energy
#1	-651.540 +/- 76.650	-8829.372 +/- 181.557	4639.679 +/- 283.160	-93.792 +/- 7.023	-4935.025 +/- 79.020
#2	-378.791 +/- 52.874	-8660.352 +/-198.607	3346.314 +/- 273.093	-68.419 +/- 4.411	-5461.248 +/- 179.771
#3	-324.959 +/- 39.065	-8887.285 +/- 243.068	3932.756 +/- 347.091	-62.877 +/- 5.524	-5342.365 +/- 123.070
Average					-5246.212 +/-275.97

Second TNP1



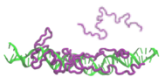
	Van der Wall Energy	Electrostatic energy	Polar solvation Energy	SASA Energy	Binding Energy
#1	-587.869 +/- 63.608	-6999.452 +/-156.402	3992.172 +/- 297.726	-73.851 +/- 6.181	-3669.001 +/- 182.723
#2	-711.902 +/- 38.798	-6996.487 +/-144.507	4092.904 +/- 279.643	-86.490 +/- 4.466	-3701.975 +/- 148.780
#3	-370.879 +/- 38.185	-7013.783 +/- 117.377	3529.047 +/- 120.218	-51.000 +/- 2.596	-3906.615 +/- 77.901
Average					-3759.197 +/-128.73

Third TNP1



	Van der Wall Energy	Electrostatic energy	Polar solvation Energy	SASA Energy	Binding Energy
#1	-486.518 +/- 84.552	-3891.004 +/- 546.778	1808.582 +/- 696.887	-61.371 +/- 10.907	-2630.310 +/- 269.427
#2	-462.690 +/- 56.904	-4932.031 +/-117.716	3494.612 +/- 216.437	-66.063 +/- 5.541	-1966.173 +/- 116.028
#3	-628.160 +/- 83.321	-5029.440 +/- 150.782	3765.859 +/- 334.494	-84.525 +/- 7.614	-1976.267 +/- 165.080
Average					-2190.92 +/- 380.56

Fourth TNP1



	Van der Wall Energy	Electrostatic energy	Polar solvation Energy	SASA Energy	Binding Energy
#1	-595.611 +/- 58.761	-3160.143 +/-87.944	2844.414 +/- 231.137	-74.129 +/- 7.279	-984.469 +/- 168.636
#2	-362.525 +/- 52.674	-2603.994 +/-113.947	2361.422 +/- 255.034	-53.504 +/- 4.802	-658.601 +/- 171.609
#3	-224.661 +/- 27.328	-2287.626 +/- 47.519	1758.375 +/- 153.874	-35.141 +/- 3.339	-789.052 +/- 134.925
Average					-810.71 +/- 164.01

Figure S4: MMPBSA energy for sequential binding of the first, second, third, and fourth TNP1 to a DNA molecule (40 bp). Solute dielectric constant is set as 8.0. Energy unit is KJ/mol.

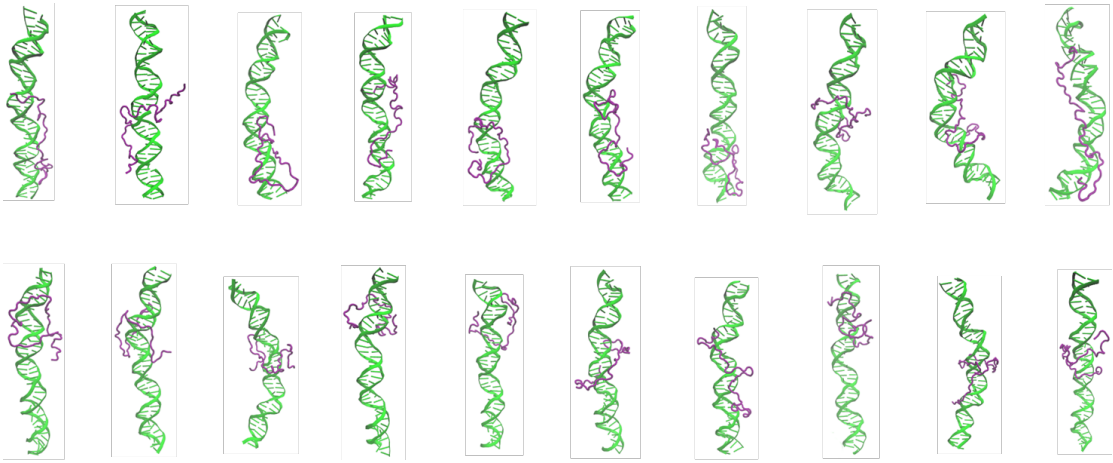


Figure S5: Final configurations of TNP1-DNA complex in 20 sets of MD simulations.

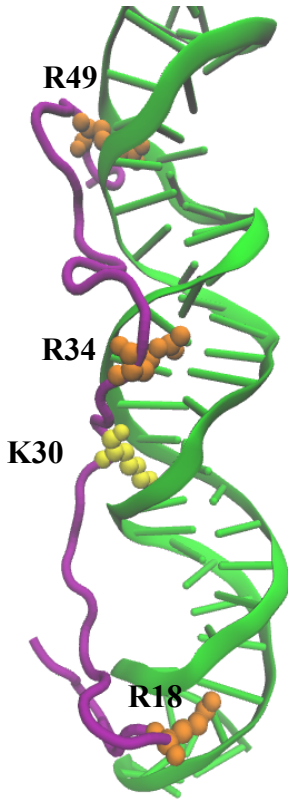


Figure S6: Depictions of arginine and lysine insertion into DNA grooves. A minimum distance threshold of 5 angstroms was utilized to determine the insertion of either the guanidinium group of arginine or the ε-amino group of lysine into the DNA groove based on their proximity to the O2 of thymine.

Figure S7: A segment (marked in purple, orange, and green) consists of arbitrary 10 connected base pairs. In each segment, the heavy atoms of the DNA backbone (adenine (A) and guanine (G) for purine bases, and thymine (T) and cytosine (C) for pyrimidine bases) are chosen, and the geometric center of the segment is calculated. The bending angle is defined as the cross angle between the vector connecting the center of $(i-1)^{th}$ and i^{th} segment and the vector connecting the center of i^{th} and $(i+1)^{th}$ segment.

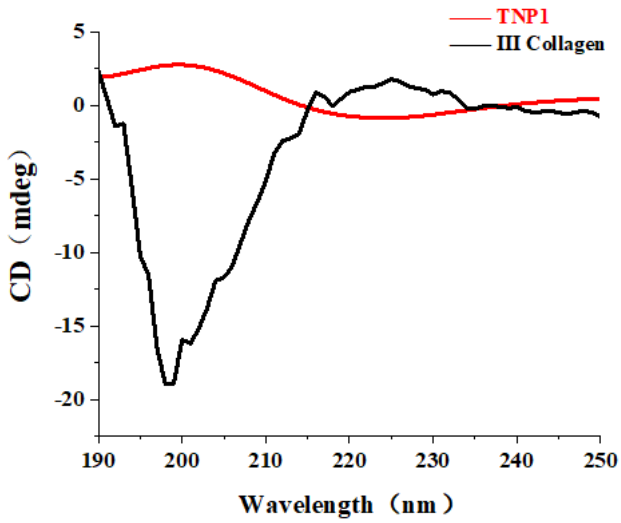


Figure S8: CD spectrum of TNP1. The signal intensity is low, and DichroWeb analysis (http://dichroweb.cryst.bbk.ac.uk/html/home.shtml) indicates that TNP1 lacks secondary structure. The black line represents a typical CD spectrum of collagen-like triple-helical secondary structure, which is characterized by a positive peak at 221 nm and a negative peak at 198 nm.

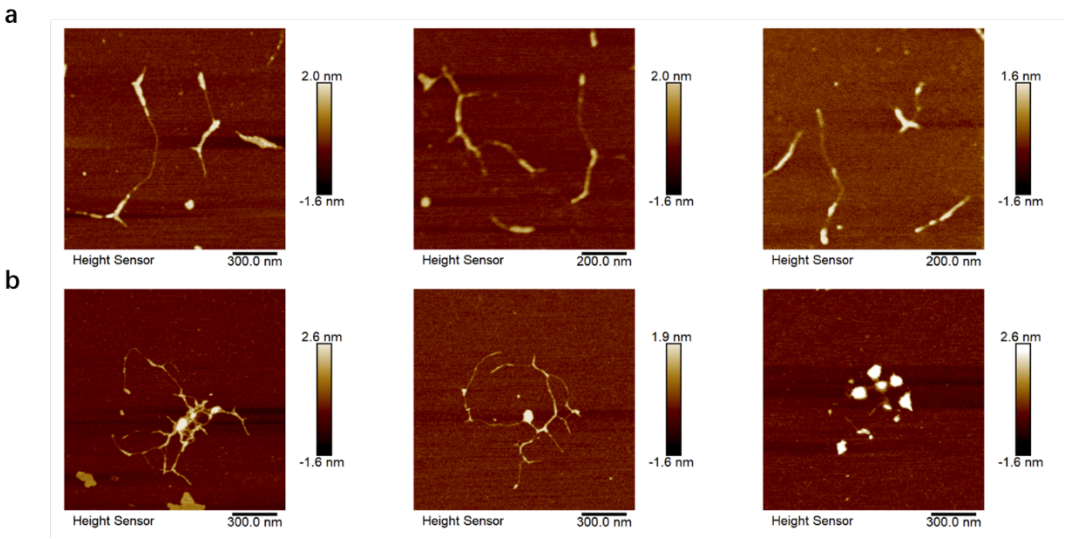


Figure S9: AFM images of (a) pure DNA (1.5 μM/bp) and (b) DNA-TNP1 complex (DNA of 0.3 μM/bp and TNP1 of 0.1 μM)

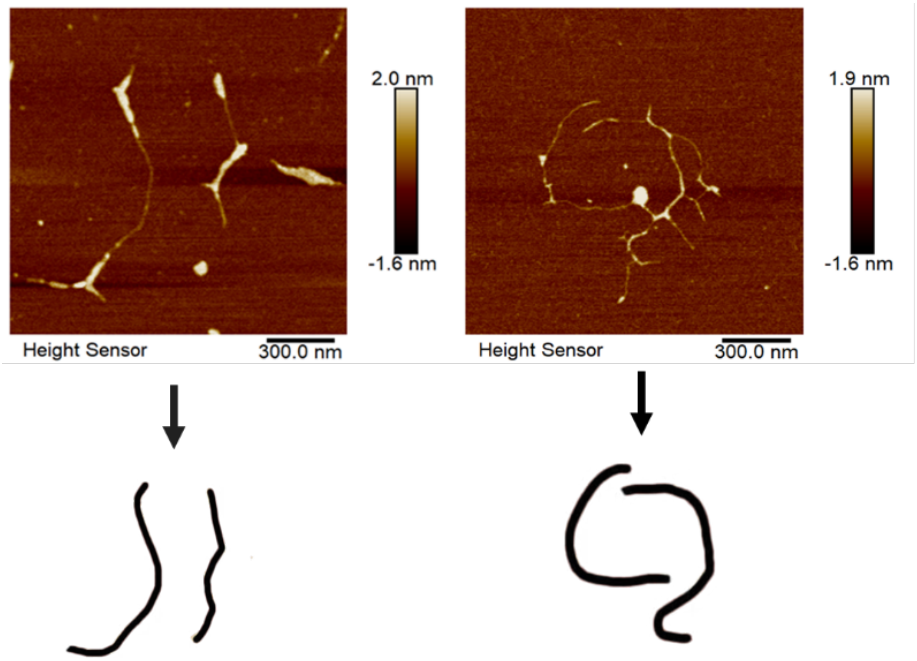
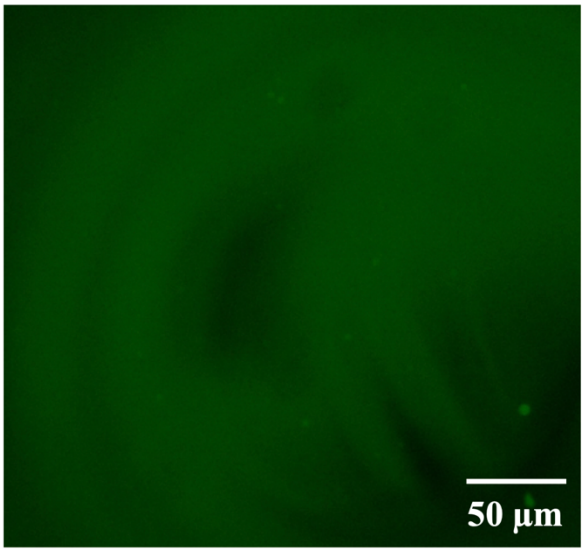


Figure S10: Extraction of DNA contour from AFM images.



5 µM eGFP-TNP1+ 120 µM/bp DNA

Figure S11: Fluorescence imaging of 120 µM/bp DNA mixing with 5 µM eGFP-TNP1.

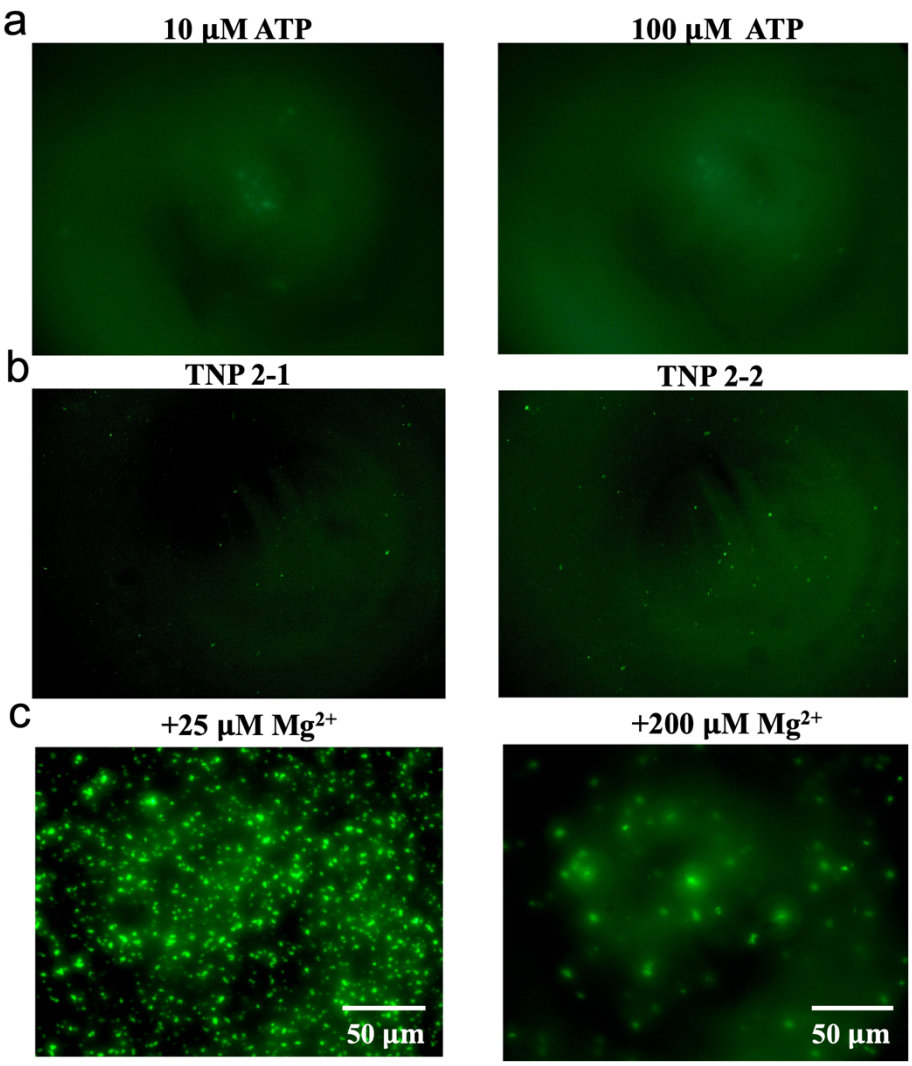


Figure S12: Fluorescence images under different experimental conditions. (a) Fluorescent images depicting the mixture of 10 µM eGFP-TNP1 with either 10 µM or 100 µM ATP. (b) Fluorescent images of the mixture of eGFP-TNP1 2-1 or eGFP-TNP1 2-2 with 177 bp DNA. Various $R_{+/-}$ ratios were measured in all cases, revealing either no or very weak phenomena of droplet formation. Two representative images are presented here. (c) Addition of 25 µM or 200 µM Mg^{2+} into preformed droplet systems of eGFP-TNP1: 177 bp DNA mixture.

Table S2: Utilization of Various DNA in the Conducted Assays

DNA Length	Assays
40 bp	Molecular Dynamics (MD) Simulation
177 bp	Gel Electrophoresis, Bio-layer Interferometry, Measurement of ζ-Potential, CD Spectrum, Dynamic Light Scattering, Fluorescence Imaging
3043 bp	Atomic Force Microscopy (AFM)