Supporting Information

The Base Pair Contents and Sequences of DNA Double Helixes Differentiated by Surface-Enhanced Raman Spectroscopy

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EXPERIMENTAL METHODS

Ag IANPs preparation

The AgIANPs was prepared based on our previous work.[1] Briefly, 5 mL of sodium citrate-reduced silver sol was centrifuged (5000 rpm, 15 min, 15 ° C), after the removal of the supernatant, 50 μL of the centrifuged silver sol was mixed with 50 μL of potassium iodide solution (1 mM) at room temperature for 25 minutes. Then, the mixture was further mixed with 50 μL DNA sample and 20 μL of Al^{3+} (0.01 M $Al_2(SO_4)_3$) one after another. After that, the mixture was shaken well for SERS detection. The SERS test instrument is made by Kaiser Optical Systems Inc. (Tokyo, Japan). The laser wavelength is 633 nm, the scan time is 10 s, and each test is accumulated twice. All DNA sequences (linear, hairpin or dsDNA) were analyzed at 1 μM concentration throughout the SERS assay unless otherwise stated.

Sample preparation for SERS

DNA strands (HPLC grade) （Table 1）were purchased from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). 100 μM DNA samples are annealed in 50 mM ammonium acetate solution to form hairpin conformations by heating them in 90 °C water bath for 10 minutes followed by cooling down to room temperature.Ultra-pure water was used throughout the experiment.

CD spectroscopy

CD spectra were recorded on a J-810 CD spectrometer (JASCO, Tokyo, Japan) using 0.2 mm path length Hellma cell at the room temperature. Spectra were recorded from 320 to 200 nm and presented as the averages of three scans. Each trace was measured at 100 nm/min of scanning speed, 1 nm data pitch and 1 nm bandwidth. The background spectra corresponding to the buffer alone were subtracted from all DNA spectra. The final DNA concentration for CD detection is 25 μM.

Normalized peak position analysis

To determine the normalization peak, we find that, between the two hairpin conformations (2HP4a and 2HP4b), the unique difference is the base pair sequence (Figure S3). Thus, we hold the hypothesis that the unusual SERS spectra of the DNA hairpins formed by the strands with the same composition and the different base sequences, may be due to the characteristic peaks normalized in an inaccurate way, as the position of phosphodiester group to the surface of silver nanoparticles varies along the helix. Fortunately, the band corresponding to the deoxyribose was detected in our SERS spectra. Inspecting the double helix conformation, the deoxyribose is wrapped inside the phosphate group and stays relatively far from the surface of silver nanoparticles. The signal of deoxyribose is less likely to have major changes by the base stacking changes, making its peak intensity a more reliable internal standard. To profoundly demonstrate the feasibility and accuracy of the SERS method to analyze the stacking effect on the SERS spectra of the DNA hairpins, we normalized the peaks of 2HP4a and 2HP4b with the intensity of deoxyribose (Figure S2B). Comparing the SERS bands of G and A ring breathing at 655 cm^{-1} and 731 cm^{-1} normalized by the phosphate band, the two band intensity normalized by deoxyribose shows less different but still distinguishable spectra.

In another sets of experiments, our hypothesis was further confirmed. Figure S4 exhibits the SERS spectra of complemented double-strand DNA, DS1-DC1, and DS1-DX1, normalized at the peak intensity of the phosphate group. It can be observed the significant increment of the ring breathing signal of guanine and adenine, clearly, after the mismatch, one certain pair of bases have muted into an adenine-guanine base pair, which is not able to form the hydrogen bond with each other. However, the peak intensity at 1703 cm^{-1} (represent the dG ν C=O H-bond) increases unusually, which

contradicts with the decrease of numbers of G-C base pairs caused by mutation. Therefore, this again proves that the peak intensity at 1089 cm^{-1} is not suitable as a perfect internal standard for signal normalization.

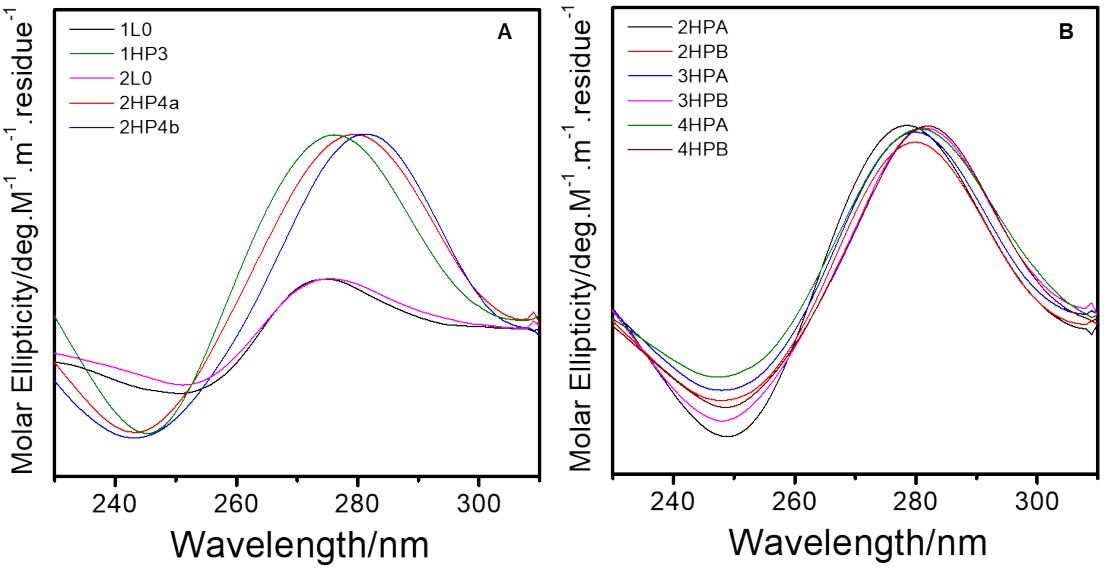

Figure S1 CD spectra of DNA strands of A) 1L0, 1HP3, 2L0, 2HP4a, 2HP4b and B) 2HPB, 2HPA, 3HPB, 3HPA, 4HPB and 4HPA annealing at 100 μM DNA concentration and 50 mM ammonium acetate and detected at 25 μM DNA concentration after sequence dilution.

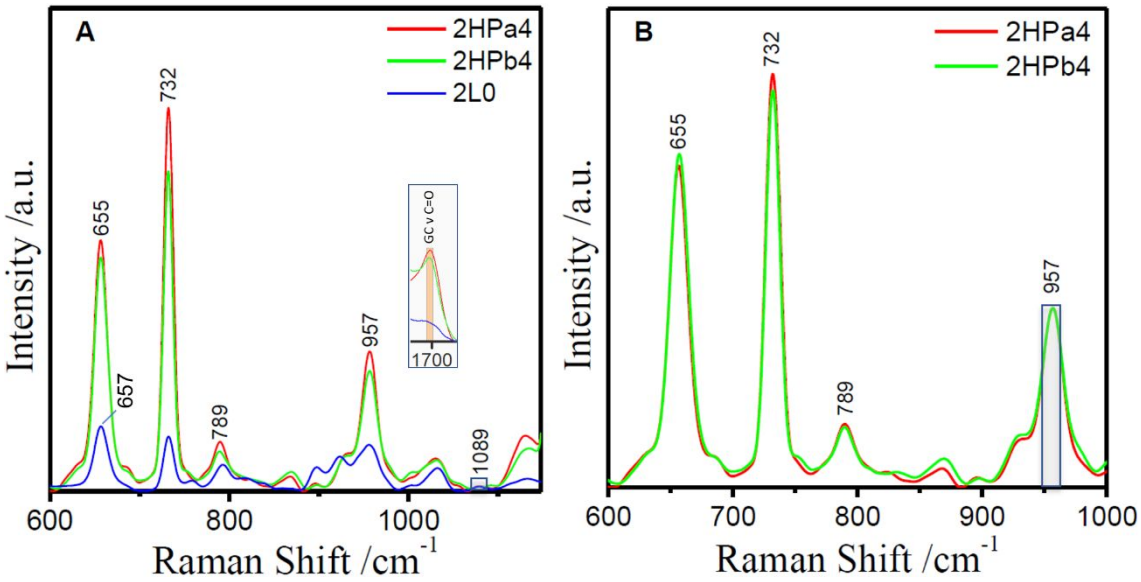

Figure S2 A)The SERS spectra of a DNA random coil 2L0 (bule line) and two distinct hairpins 2HP4a (red line) and 2HP4b (green line).Above SERS spectra normalized by the intensity of phosphate band at 1089 cm^{-1} ; B)The SERS spectra of 2HPa4 and2HPb4 (normalized by the intensity of deoxyribose band at 957 cm^{-1}.

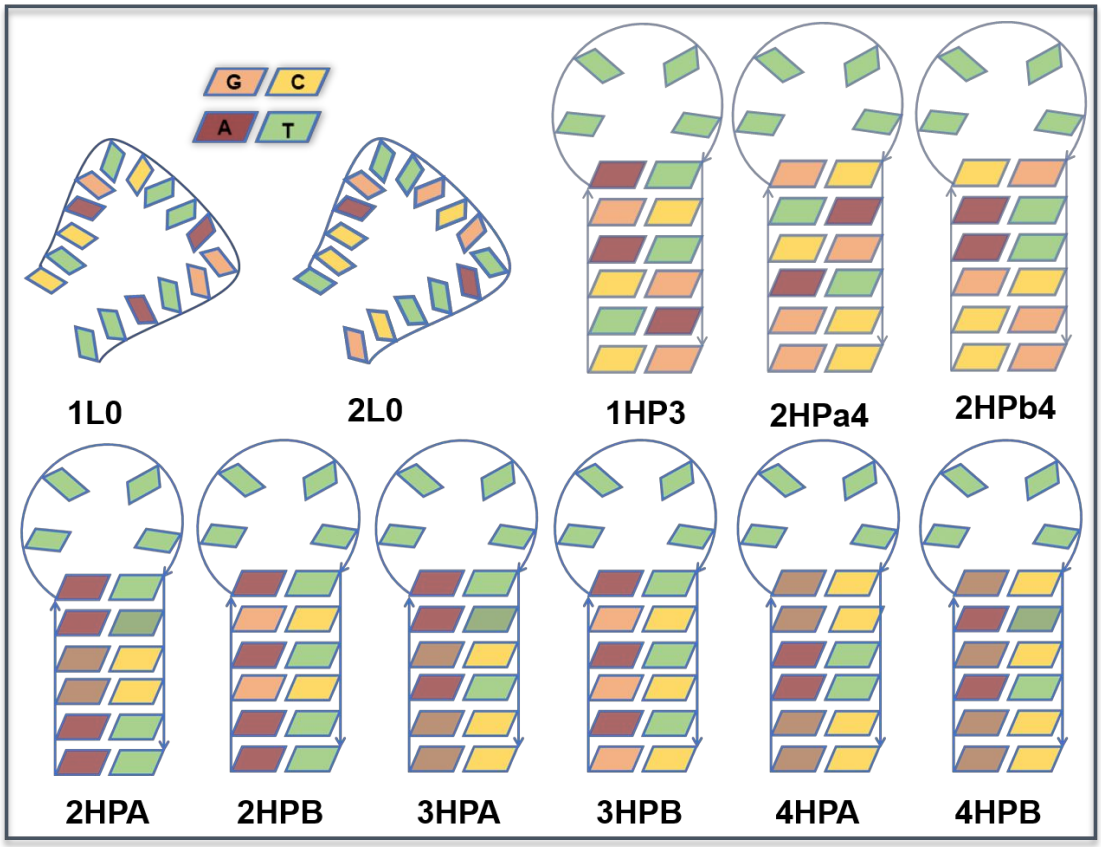

Figure S3 Schematic diagram of 1L0, 2L0 and nine hairpin structures formed by 1HP3, 2HPa4, 2HPb4, 2HPA, 2HPB, 3HPA, 3HPB, 4HPA and 4HPB.

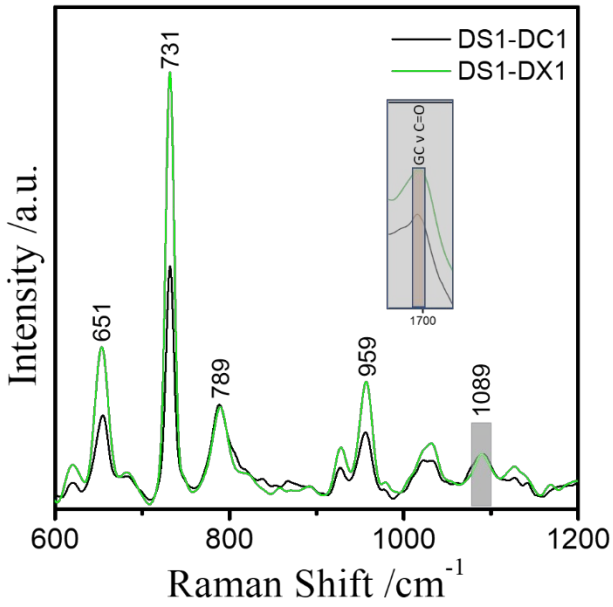

Figure S4. The SERS spectra of DS1-DC1 and DS1-DX1 double helixes normalized at the peak intensity of phosphate group at 1089 cm^{-1}

Table S1. The bands and their assignment appeared in the SERS spectra of DNA hairpin$[1HP3\&1L0]_4$

Raman Shift/cm^{-1}		Assignment		Ref.
1L0	1HP3	ss DNA	DNA hairpin	
571	570	dG	dG C2'-endo/*anti*	[2, 3, 4]
-	620	-	Loop	[3,4]
654	651	dG R6 ring br	dG ring br,	[2, 3, 5]
732	732	dA	dA ring br	[3, 4, 5]
789	788	dC	dC ring br	[3, 4, 5]
794	798	ν_s OPO	ν_s OPO; dC ring br	[2, 4, 5]
860	864	ν OPO	ν OPO, bk	[2, 3, 4]
925	929	d	bk	[2, 3, 4]
951	959	d	d, dG δ R5	[3, 4, 5]
1033	1031	d (ν C-O)	d (ν C-O)	[3, 4, 5]
1089	1089	ν_s PO_2^-	ν_s PO_2^-, bk	[2, 3, 4]
1184	1220	dT	dT C2'-endo/*anti*	[2, 4]
1251	1247	dT, dG	dT, dG δ NH(N2)	[4, 5]
1324	1324	dG	dG C2'-endo/*anti*	[3, 4]
-	1337	-	dG C2'-endo/*syn*	[3, 4]
-	1385	-	dG C2'-endo/*syn*	[4, 5]
-	1585	-	dG δ NH (N2H H-bond)	[3, 4]
1650	1660	dT	dT, dC ν C5=C6	[3, 4, 5]

-	1703	-	dG ν C=O H-bond	[3, 4]

Note: d = deoxyribose, ν = stretch, δ = deformation, br = breathing, s = symmetric, s = shoulder, bk = backbone.

References

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