Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Tip Enhanced Raman Spectroscopy: Plasmid-Free vs. Plasmid-Embedded DNA

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Preparation of gold nanoplate substrates

Synthesis of gold (111) nanoplates was carried out using the method reported by Pashaee et al.[1] based on the initial work of Chu et al.[2] Once large gold nanotriangles were obtained, the solution was transferred to an amine-coated test tube for further purification of the gold nanoplates by centrifugation (4000 rpm for 10 min for 5–6 times). The test tubes used for centrifugation were coated with aminopropyltrimethoxysilane (APTMS) by evaporation at 130°C. Trapping of the gold nanoplates on the side walls of the test tubes was effective, preventing their aggregation at the bottom of the tube centrifugation. The gold nanoplates were subsequently released by sonication followed by drop-casted onto clean Quartz coverslips (120 mm thickness). AFM characterization of individual nanoplates shows roughness in the range of about 500 pm. The full TERS setup is illustrated below in Fig S1.

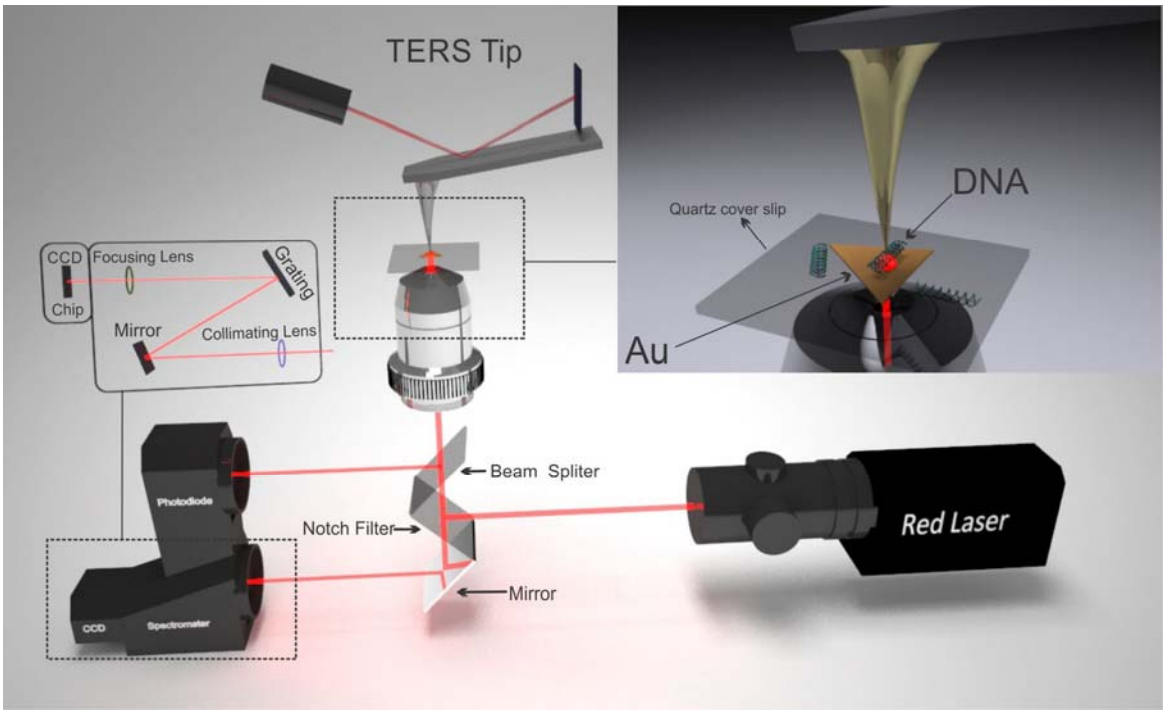

Fig. S1 Schematic illustration of full TERS setup and sample configuration.

Complete vibrational TERS fingerprint assignments for the plasmid-free DNA (Table S1) and plasmid-embedded DNA (Table S2) have been shown as follows:

Table S1.TERS chemical assignment of plasmid-free DNA in four different spots; Raman modes: v (stretching), δ (bending), S (symmetric). Abbreviations: H (high), M (medium), L (low), **A** (Adenine), **C** (Cytosine), **G** (Guanine), **T** (Thymine)[3-12]

1	2	3	4	Assignment (plasmid-free DNA)	Ref
665 L		670 L	672 L	**G** (ring breathing)	[3, 4]
	687 L	683L	699 L	**C** C_5C_4-N_3C_4	[11]
709 L	709 L		716 L	**A** (in-plane ring breathing)	[3,5]
		730 M	738 L	**A** Ring stretching	[11]
762 L	742 L			**T** Ring breathing	[3,6,7]
		773 L	770 M	**T** Ring breathing	[11]
			782 M	**C** Ring breathing	[8]
		802 L	806 M	Tris-HCl	[5]
		855 L	851 L	**G** N_7C_5-$N_1C_2N_3$	[11]
869 L				Deoxyribose ring	[3,8]
		906 L		**A/C/G** ρ (NH_2) NH_2 Rocking; Deoxyribose	[3,4,8]
940 L	936 L			**A/C/G** δ (NH_2) NH_2 Rocking + δ(C–H) + δ(ring)	[3, 4, 6]
974L				**T** ν_s (C-C), ν_s (C-O), ribose	[3, 9]
998L			997 L	**T** Out-of-plane δ (NH_2) wagging	[3,6]
	1020 M			C NH_2 + C_6-H	[11]

	1028 M		1028 L	**A** ν_s(N-C) N-Sugar Stretching; **A** $NH_2 + N_9$-H	[3,4,11]
1043 L		1042 L	1046 L	**T** Out-of-plane $\delta(CH_3)$ wagging	[6]
1113 M		1099 L	1089 L	PO_2	[3]
		1122 M	1124 M	**C** $\nu(C_5C_6–C_6N_1) + \delta(C_5H)$ in- plane; **A** $N_3C_2 + N_9$-H	[6,11]
	1131 L			**A** $\nu(C_8–N_9)$, $\delta(N_9–H, C_8–H)$	[3,6]
	1166 L	1164 M		**A/G** $\nu_s(C_5$-$C_6)$ C-C Stretching	[3,4]
	1196 M			**C**	[3,12]
1210 H	1208 H		1202H	**T** ν_s(C-C) Ring-CH_3 Stretching	[3,4,9]
		1216 M		**T** in-plane $\nu_s(C–CH_3)$	[3,6]
1246 H	1256 H	1246 M	1240 H	**A** $\delta(C_8–H, N_9–H)$, $\nu(N_7–C_8)$; **C** ν_s(C-C) Ring-CH_3 Stretching **G** $\nu_s(C_8–N_9)$ C-N Stretching; **T** In-plane ν(ring)	[3,4,6,7]
1274 H		1278 H		**C** $\nu(C–NH_2)$ + in-plane ν(ring); **T** Ring + CH; **G** C_8N_7-$N_1C_6 + N_7C_5$	[3,6,11]
	1296 M		1291 H	**C** $\nu_s(C_2$-$N_3)$ C-N Stretching	[3,4,10]
		1314 M		**A** $\nu(C_2–N_3, N_1–C_2, C_5–C_6, C_5–N_7)$; **G** ν_s (C-N) C-N Stretching (Im)	[4,6]
1324 H			1336 L	**A/G** Ring mode	[3,8]
1346 H	1345 H	1353 H		**T** N_3H-C_4=O	[11]
			1359 M	**A/C/T/G** ν_s (C-N) C-N Stretching (py)	[3,4]
1390 H	1403 H		1398 L	**T** δ(NH) deformation $\delta(CH_3)$ CH_3 deformation	[3,4,6]
1413m				**A** $\delta(C_2–H, N_9–H)$, $\nu(C_8–N_9, C_4–N_9)$; **C** $\nu_s(C_4$-$C_5)$ C-C Stretching; **T** δ(NH) + in-plane ν(ring)	[3,4,6]
		1433 H	1423 H	C	[11]
1451 L		1455 L		**A** C_2H-$N_1C_2 + N_3C_2$ / **G** N_1C_2-N_1C_6	[11]
	1468 H	1473 L	1467 H	**A** ν_s (C=N) C=N Stretching (Py); **T** $–N_1C_2 + C_2N_3$	[3,4,11]
1499m				**G** ν_s (C=N) C=N Stretching (Im)	[3,4]
		1509 H	1527 H	**C** $\delta(NH_2)$ NH_2 Deformation; **G** C_4C_5-C_4N_9	[3,4,11]
1538 H		1547 L		T in-plane ring stretching	[3,6]
	1578 H	1577 H	1573 M	**A/C/G/T** Ring Stretching (Py)	[3,4]
1602 H	1605 H	1603 L	1592 M	**A/C/G** $\delta(NH_2)$ NH_2 Deformation	[3,4,6,11]
1672 L			1669 L	**A** $\beta_s(NH_2)$ NH_2 Scissoring	[3,4]
		1683 M		**G** C_6=O + C_5C_6	[11]

Table S2.TERS chemical assignment of plasmid-embedded DNA in four different spots; Raman modes: ν (stretching), δ (bending), S (symmetric). Abbreviations: H (high), M (medium), L (low), **A** (Adenine), **C** (Cytosine), **G** (Guanine), **T** (Thymine)[3-12]

1	2	3	4	Assignment (Plasmid-Embedded DNA)	Ref
729 L	729 L	716 L		**A** (in-plane ring breathing)	[3, 5]
743 L	741 L	749 L	772 L	**T** Ring breathing	[3,6,7]
782 L				**T** Ring breathing	[11]
816 H	802 L	813 L	804 L	**T** N_1C_2+N_1--H+C_5C_4+N_1C_6+N_3C_4; Tris-HCL	[5,11]
847 L		844 L		**G** N_7C_5-$N_1C_2N_3$	[11]
	877 L	869 L	884 L	Deoxyribose ring	[3, 8]
942 H		937 M		**A/C/G** $\delta(NH_2)$ NH_2 Rocking + δ (C–H) + δ (ring)	[3, 4, 6]

960 H		960 M		**C** δ (NH) out-of-plane wagging	[6]
		976 L		**T** ν_s (C-C) ν_s (C-O), and ribose	[3, 9]
980 L		986 L		**C** C_5H	[11]
1031 L	1039 L	1026 L		**A** ν_s(N-C) N-Sugar Stretching; **A** ν (NH_2) + N_9-H; **C** ν (Ring) + δ (C–H) in-plane	[3,4,6,11]
		1077 L	1072 L	G	[3,4]
1122 M	1118 H	1114 M	1124 M	**C** ν (C_5C_6–C_6N_1) + δ (C_5H) in-plane; **A** N_3C_2 + N_9-H	[6,11]
	1133 M	1133 M	1144 M	**A** ν (C_8–N_9), δ (N_9–H, C_8–H)	[3,6]
1157 H		1155 M		**G** C_8N_7 + N_9H-C_4N_3	[11]
	1164 H	1165 M	1174 L	**A/G** ν_s (C_5-C_6) C-C Stretching	[3, 4]
		1191 L	1195 M	C	[3,12]
		1207 L		**T** ν_s(C-C) Ring-CH_3 Stretching	[3,4,9]
1216 H		1219 L		**T** in-plane ν_s(C–CH_3)	[3,6]
1235 H		1230 H	1230 L	C	[11]
1259 L	1252 M	1249 L	1264 L	**A** δ (C_8–H, N_9–H), ν(N_7–C_8); C ν_s(C-C) Ring-CH_3 Stretching **G** ν_s(C_8–N_9) C-N Stretching; **T** In-plane ν(ring)	[3,4,6,7]
1275 L	1278 L	1286 H		**C** ν_s(C–NH_2) + in-plane ν_s(ring); **T** Ring + CH ; **G** C_8N_7-N_1C_6 + N_7C_5	[3,6,11]
1296 L	1296 H		1303 M	**C** ν_s (C_2-N_3) C-N Stretching	[3,4,10]
	1324 L	1312 H		**A** ν (C_2–N_3, N_1–C_2, C_5–C_6, C_5–N_7); G ν_s (C-N) C-N Stretching (Im)	[4,6]
		1337 H	1328 L	**A/G** Ring mode	[3,8]
1344 H			1343 L	**T** N_3H-C_4=O	[11]
				G νs (C-N) C-N Stretching (py)	[4]
	1362 H	1370 L	1370 L	**A/C/T/G** ν_s (C-N) C-N Stretching (py)	[3,4]
1383s			1386 L	**G** C_2N_3-C_2	[11]
		1396 H		**T** δ (NH) deformation δ (CH_3) CH_3 deformation	[3,4,6]
1427 H		1432 L	1417 M	**C**	[11]
	1443 M		1443 L	**T** C_5-Me	[11]
1461 L				**A** ν_s(C=N) C=N Stretching (Py); C_2H-N_1C_2 + N_3C_2; G N_1C_2-N_1C_6	[4,11]
	1477 L			**A** ν_s (C=N) C=N Stretching (Py); **T** –N_1C_2 + C_2N_3	[3,4,11]
1488 H			1488 L	**C** N_1C_6 + N_3C_4	[11]
	1511 H	1509 H	1517H	**C** δ (NH_2) NH_2 Deformation; **G** C_4C_5-C_4N_9	[3,4,11]
1543 L	1546 H	1541 H	1539 L	**T** in-plane ring stretching **A** Ring Stretching (Py)	[3,4]
1563 M	1563 H	1565 H	1560 H	**A/C/G/T** Ring Stretching (Py)	[3,4]
1581 L			1580 H	**A** δ (NH_2) NH_2 Deformation; **C** C_4C_5-C_5C_6; **T** N_3C_4+N_1C_2+C_6C_5-N_1C_6; **G** N_3C_4-C_4C_5	[4,11]
1596 L	1596 L	1591 H	1609 L	**A/C/G** δ (NH_2) NH_2 Deformation	[3,4,6,11]
	1641 L	1632 M	1637 M	**C/G/T** ν_s (C=O) , ν_s (C=C)	[3,4,9]
		1659 L		**T** C_4=O + C_5-C_6	[11]

1666 L			**A** $\beta_s(NH_2)$ NH_2 Scissoring	[3,4]
	1689 M	1682 L	**G** C_6=O + C_5C_6	[11]

Plasmid-free and Plasmid-embedded DNAs' distinct nanoscale morphologies

In order to evaluate the reproducibility of the observed nanoscale morphologies of both plasmid-free and plasmid-embedded DNAs deposited on quartz coverslips, AFM images were obtained on three samples and multiple spots of each sample. More representative AFM images showing the nanoscale morphologies of these two types of DNAs have been demonstrated in Fig. S2. As shown, the plasmid-free DNAs tend to generate a more conglomerated structure as opposed to plasmid-embedded DNAs showing more linear anisotropic orientation. This is also in good agreement with the structure of plasmid-embedded DNAs since the DNA insert is more tightly tethered by plasmid into its supercoil structure as opposed to plasmid-free DNA. Since the DNA insert is not limited to plasmid in the plasmid-free DNA structure, these DNAs tend to more randomly aggregate with one another as shown in Fig. S2.

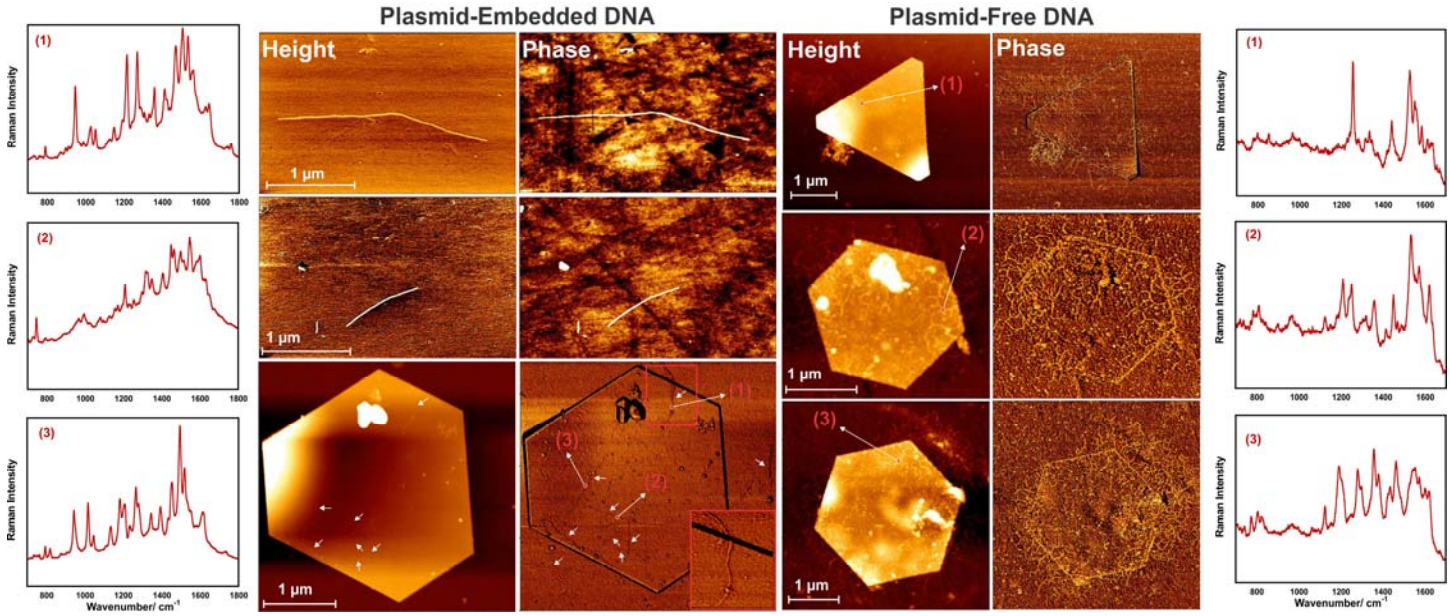

Fig. S2. AFM images of plasmid-free and plasmid-embedded DNA representing their distinct morphologies and the representative TERS fingerprints on the selected regions shown on AFM images.

TERS fingerprint of both types of DNAs on quartz

The TERS fingerprints of both studied types of DNAs on quartz are not clearly observable on the same Raman intensity scale used for DNA signals obtained on gold nanoplates. Therefore, three different representative signals are shown separately for each type of the DNAs in Fig. S3.

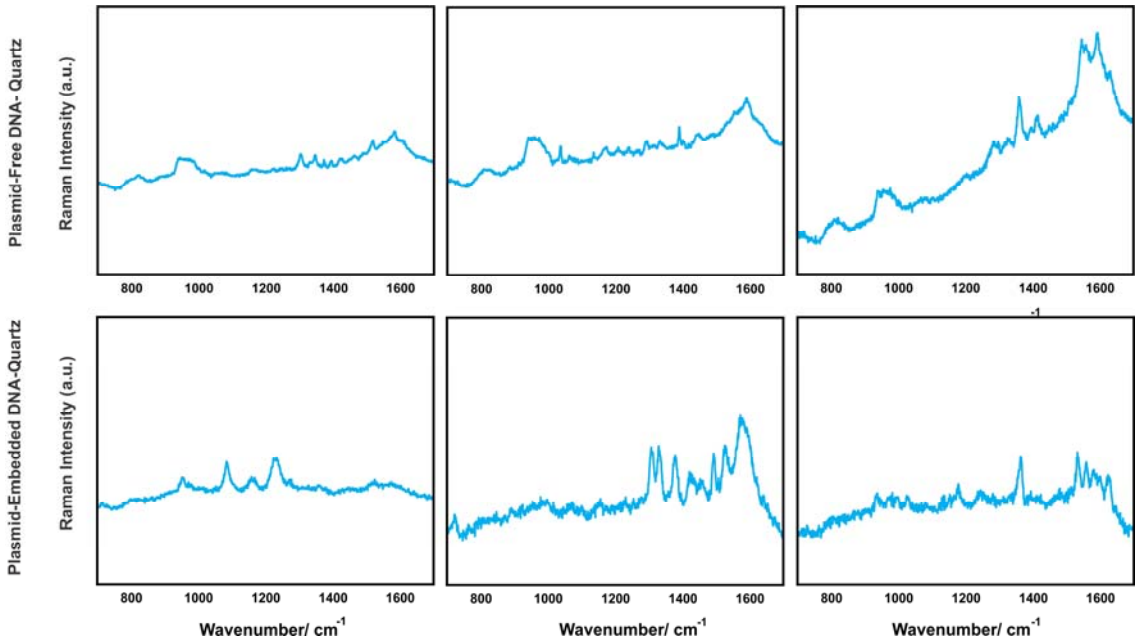

Fig. S3. TERS fingerprint of plasmid-free and plasmid-embedded DNAs on quartz at distinct positions. The scale used in these spectra is multiplied by a 20 fold factor compared to the TERS spectra acquired in gap mode condition shown in Fig S2.

DNA preparation and purification

β_2-adrenergic receptor (β_2AR)-Flag tagged plasmid was digested using HindIII and XbaI enzymes from the FastDigest kit (Life Technologies). About 5µg of DNA was incubated with both enzymes at 37˚C for 20 minutes. Control pcDNA1.1 plasmid without the β_2AR-Flag insert was also digested using the same conditions. The digested fragments were subjected to agarose gel electrophoresis (0.7% w/v). The DNA was stained using RedSafe (FroggaBio) and the band relative to the β_2AR-Flag was extracted under UV light. The expected size for the human β_2AR DNA plus the Flag tag is about 1.1 Kb. The expected size for the pcDNA1.1 plasmid is 4.8 Kb. GeneRuler 1Kb plus DNA ladder was used as a reference.

The β_2AR-Flag band was purified using a gel extraction kit (Qiagen). Some of the purified DNA was subjected to agarose gel electrophoresis (0.7% w/v) and compared to the non-digested original plasmid

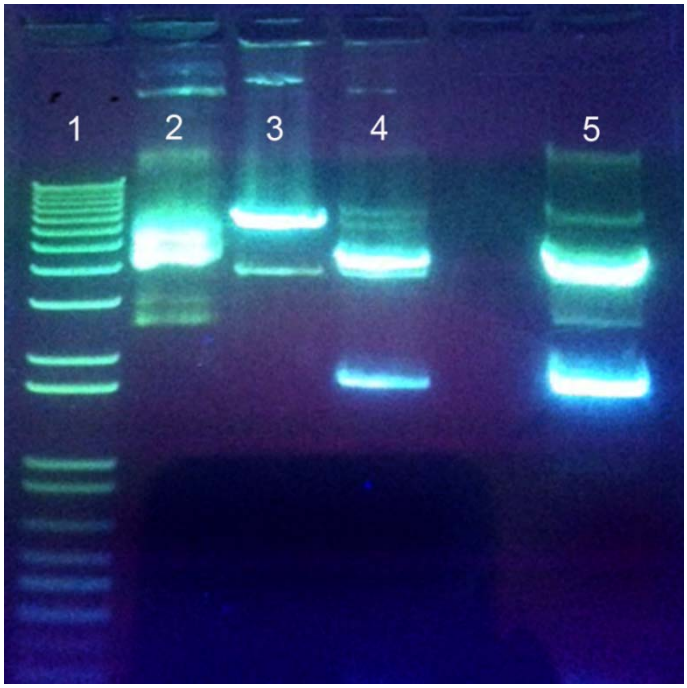

Fig. S4 Lanes = 1) Kb plus ladder 2) non digested plasmid 3) plasmid digested with HindIII 4) plasmid digested with XbaI 5) plasmid digested with both enzymes

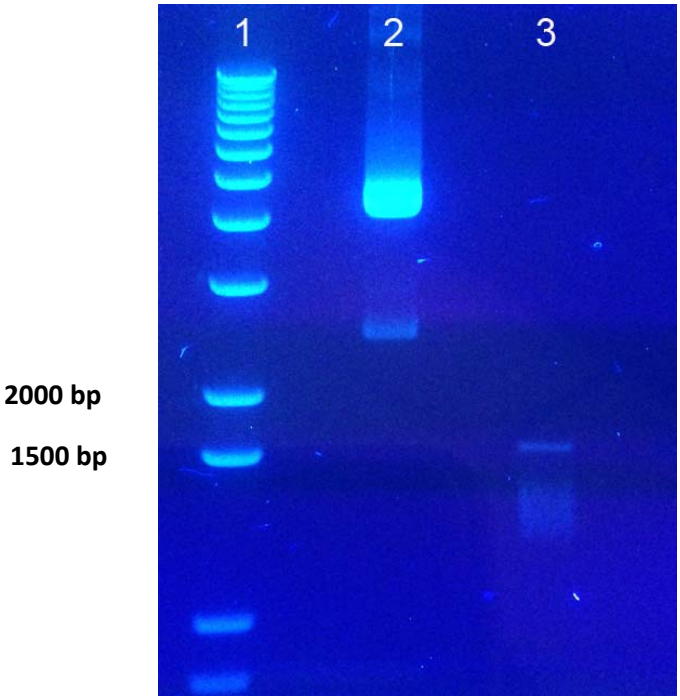

Fig. S5 Lanes = 1) Kb plus ladder 2) purified double digested plasmid

CONTROL: pcDNA1.1 purified

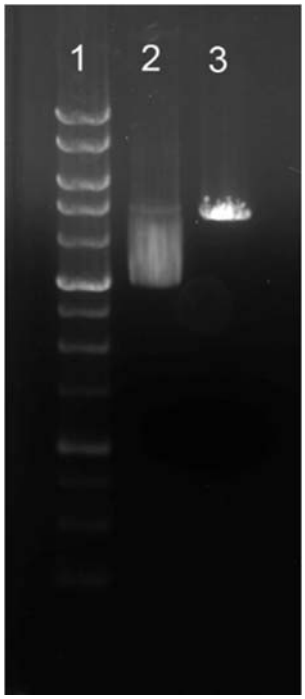

Fig. S6 Lanes = 1) Kb plus ladder 2) non digested pcDNA1.1 3) double digested and purified pcDNA1.1

DNA insert sequence (1239 base pairs)

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1551 CCCCCAGCCA GTGCGCTTAC CTGCCAGACT GCGCGCCATG GGGCAACCCG
                                          M   G  Q  P  G   Frame 1

1601 GGAACGGCAG CGCCTTCTTG CTGGCACCCA ATAGAAGCCA TGCGCCGGAC
       N  G  S   A  F  L   L  A  P  N   R  S  H   A  P  D    Frame 1

1651 CACGACGTCA CGCAGCAAAG GGACGAGGTG TGGGTGGTGG GCATGGGCAT
     H  D  V  T   Q  Q  R   D  E  V   W  V  V  G   M  G  I   Frame 1

1701 CGTCATGTCT CTCATCGTCC TGGCCATCGT GTTTGGCAAT GTGCTGGTCA
      V  M  S   L  I  V  L   A  I  V   F  G  N   V  L  V  I   Frame 1

1751 TCACAGCCAT TGCCAAGTTC GAGCGTCTGC AGACGGTCAC CAACTACTTC
       T  A  I   A  K  F   E  R  L  Q   T  V  T   N  Y  F    Frame 1

1801 ATCACTTCAC TGGCCTGTGC TGATCTGGTC ATGGGCCTGG CAGTGGTGCC
     I  T  S  L   A  C  A   D  L  V   M  G  L  A   V  V  P   Frame 1

1851 CTTTGGGGCC GCCCATATTC TTATGAAAAT GTGGACTTTT GGCAACTTCT
      F  G  A   A  H  I  L   M  K  M   W  T  F   G  N  F  W   Frame 1

1901 GGTGCGAGTT TTGGACTTCC ATTGATGTGC TGTGCGTCAC GGCCAGCATT
       C  E  F   W  T  S   I  D  V  L   C  V  T   A  S  I    Frame 1

1951 GAGACCCTGT GCGTGATCGC AGTGGATCGC TACTTTGCCA TTACTTCACC
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     E  T  L  C   V  I  A   V  D  R   Y  F  A  I   T  S  P    Frame 1

2001 TTTCAAGTAC CAGAGCCTGC TGACCAAGAA TAAGGCCCGG GTGATCATTC
      F  K  Y   Q  S  L  L   T  K  N   K  A  R   V  I  I  L   Frame 1

2051 TGATGGTGTG GATTGTGTCA GGCCTTACCT CCTTCTTGCC CATTCAGATG
       M  V  W   I  V  S   G  L  T  S   F  L  P   I  Q  M    Frame 1

2101 CACTGGTACC GGGCCACCCA CCAGGAAGCC ATCAACTGCT ATGCCAATGA
     H  W  Y  R   A  T  H   Q  E  A   I  N  C  Y   A  N  E   Frame 1

2151 GACCTGCTGT GACTTCTTCA CGAACCAAGC CTATGCCATT GCCTCTTCCA
      T  C  C   D  F  F  T   N  Q  A   Y  A  I   A  S  S  I   Frame 1

2201 TCGTGTCCTT CTACGTTCCC CTGGTGATCA TGGTCTTCGT CTACTCCAGG
       V  S  F   Y  V  P   L  V  I  M   V  F  V   Y  S  R    Frame 1

2251 GTCTTTCAGG AGGCCAAAAG GCAGCTCCAG AAGATTGACA AATCTGAGGG
     V  F  Q  E   A  K  R   Q  L  Q   K  I  D  K   S  E  G   Frame 1

2301 CCGCTTCCAT GTCCAGAACC TTAGCCAGGT GGAGCAGGAT GGGCGGACGG
      R  F  H   V  Q  N  L   S  Q  V   E  Q  D   G  R  T  G   Frame 1

2351 GGCATGGACT CCGCAGATCT TCCAAGTTCT GCTTGAAGGA GCACAAAGCC
       H  G  L   R  R  S   S  K  F  C   L  K  E   H  K  A    Frame 1

2401 CTCAAGACGT TAGGCATCAT CATGGGCACT TTCACCCTCT GCTGGCTGCC
     L  K  T  L   G  I  I   M  G  T   F  T  L  C   W  L  P   Frame 1

2451 CTTCTTCATC GTTAACATTG TGCATGTGAT CCAGGATAAC CTCATCCGTA
      F  F  I   V  N  I  V   H  V  I   Q  D  N   L  I  R  K   Frame 1

2501 AGGAAGTTTA CATCCTCCTA AATTGGATAG GCTATGTCAA TTCTGGTTTC
       E  V  Y   I  L  L   N  W  I  G   Y  V  N   S  G  F    Frame 1

2551 AATCCCCTTA TCTACTGCCG GAGCCCAGAT TTCAGGATTG CCTTCCAGGA
     N  P  L  I   Y  C  R   S  P  D   F  R  I  A   F  Q  E   Frame 1

2601 GCTTCTGTGC CTGCGCAGGT CTTCTTTGAA GGCCTATGGG AATGGCTACT
      L  L  C   L  R  R  S   S  L  K   A  Y  G   N  G  Y  S   Frame 1

2651 CCAGCAACGG CAACACAGGG GAGCAGAGTG GATATCACGT GGAACAGGAG
       S  N  G   N  T  G   E  Q  S  G   Y  H  V   E  Q  E    Frame 1

2701 AAAGAAAATA AACTGCTGTG TGAAGACCTC CCAGGCACGG AAGACTTTGT
     K  E  N  K   L  L  C   E  D  L   P  G  T  E   D  F  V   Frame 1

2751 GGGCCATCAA GGTACTGTGC CTAGCGATAA CATTGATTCA CAAGGGAGGA
      G  H  Q   G  T  V  P   S  D  N   I  D  S   Q  G  R  N   Frame 1

2801 ATTGTAGTAC AAATGACTCA CTGCTGTAAA GCAGTTTTTC TACTTTTAAA
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Tip annealing process:

After deposition of 5 nm Ti and 30 nm of Au, the tip was annealed for 30 min at 180°C. The SEM images of non-annealed and annealed tips are shown as follows:

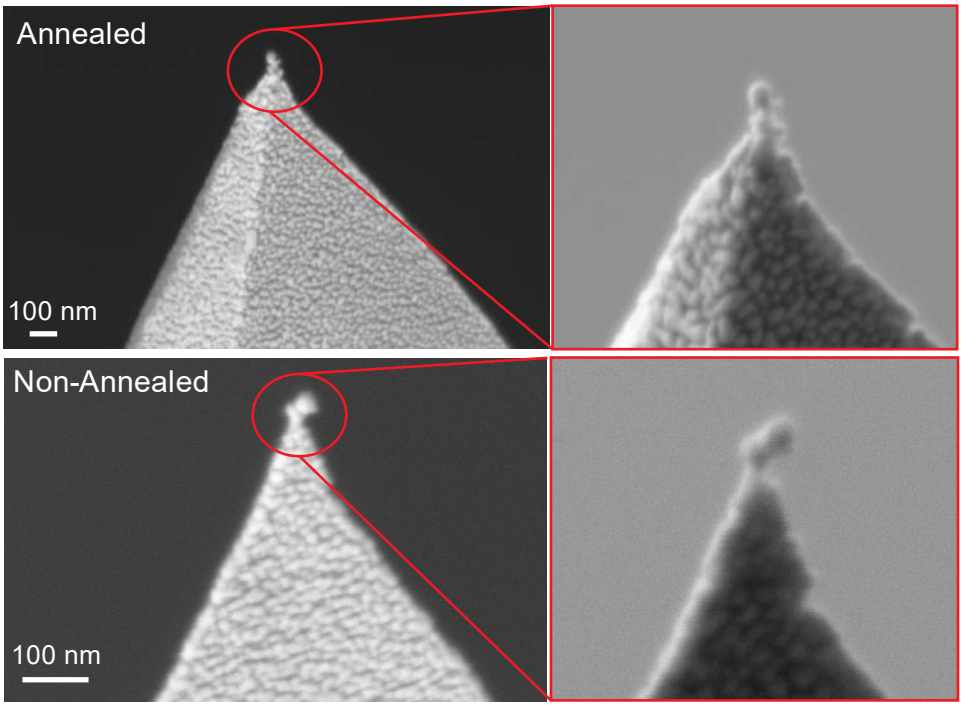

Fig. S7 SEM images showing the effect of annealing on TERS tip's shape.

Estimation of enhancement factor of TERS measurements

An estimated enhancement factor (EF) of a TERS experiment can be obtained by considering the optical conditions as well as the intensities of Raman signals when TERS tip is in proximity and away from the sample. First, the contrast was determined by equation 1:[1]

$$C = \frac{I_{TERS} - I_0}{I_0}$$

where I_0 and I_{TERS} represent the intensity of the Raman signal with the tip away and in proximity with the sample, respectively. Second, the overall enhancement factor also depends on the ratio of the

illuminated areas in the far-field (~d_L^2) and the near-field (~d_{tip}^2) conditions. In this context, EF can then be estimated by equation 2:[1]

$$EF = C \times \frac{d_L^2}{d_{tip}^2}$$

where $d_L \cong 500$ nm and $d_{tip} \cong 35$nm are the diameters of the laser spot and the tip, respectively. Contrast factors are calculated using a strong peak at 1511 cm^{-1} obtained for cytosine and guanine, C (1511cm^{-1}) = 309 where I_{TERS} = 4948.58 and I_0 = 15.96. This provides a crude enhancement factor of 6.3×10^5. Although these are crude approximations they are comparable to the distribution of TERS enhancement values reported in the literature.[1]

References:

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