## Supporting Information

# Cavity-type DNA Origami-based Plasmonic Nanostructures for Raman Enhancement 

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Figure S1. Schematic illustration and AFM images of cavity-type DNA origami with two (a), four (b), six (c), eight (d) capture strands. The scale bars are 100 nm .


Figure S2. TEM images of AuNPs-dimers based on the rectangular DNA origami and cavity-type DNA origami which carring two (a, b), four (c, d), six (e, f) and eight (g, h) capture strands with the schematic illustration and AFM images of the templates. The scale bars are 200 nm .


Figure S3. Gel electrophoresis and analysis of bands by the software of Gene Tools from Syngene were used to qualify the yields of dimer-AuNPs. Each gel image contained three lanes. One was the DNA-AuNPs as the control. The other two lanes showed the shift of the dimer-AuNPs on the template of rectangular DNA origami and cavity-type DNA origami with different number of capture strands. For the two capture strands on the template of rectangular DNA origami and cavity-type DNA origami, the yields of dimer-AuNPs were 38.701 vs 51.794 (a). For the four capture strands on the template of rectangular DNA origami and cavity-type DNA origami, the yields of dimer-AuNPs were 50.392 vs 59.030 (b). For the six capture strands on the template of rectangular DNA origami and cavity-type DNA origami, the yields of
dimer-AuNPs were 59.762 vs 76.391 (c). For the eight capture strands on the template of rectangular DNA origami and cavity-type DNA origami, the yields of dimer-AuNPs were 73.707 vs 73.817 (d).


Figure S4. Schematic illustration and AFM images of DNA origami with or without four cavities. The scale bars are 100 nm .


Figure S5. Schematic illustration and TEM images of rectangular (a) and cavity-type
(b) DNA origami based tetramer-AuNPs. The scale bars are 100 nm .


Figure S6. Gel electrophoresis and analysis of bands by the software of Gene Tools from Syngene were used to qualify the yields of tetramer-AuNPs. The image contained three lanes. One was the DNA-AuNPs as the control. The other two lanes showed the shift of the tetramer-AuNPs on the template of rectangular DNA origami and cavity-type DNA origami with different number of capture strands. For the six capture strands on the template of rectangular DNA origami and cavity-type DNA origami, the yields of tetramer-AuNPs were 21.376 vs 44.701 .


Figure S7. UV-vis absorption of 30 nm AuNPs, dimer-AuNPs and tetramer-AuNPs. The UV-vis absorption of 30 nm AuNPs indicated one well-resolved plasmon peaks in 525 nm . The UV-vis spectrum of dimer-AuNPs displayed a red shift to 527 nm which was accompanied by a small peak at $650 \mathrm{~nm} .{ }^{2}$

Calculations of the SERS enhancement factor (EF) of 4-MBA-modified NPs at the hotspots of dimers and tetramer are as follows ${ }^{1}$ :

Our pre-prepared concentration of NPs was 4.32 nM .

The surface area of each 30 nm AuNP was calculated as follows: $S=\pi D^{2}=2826 \mathrm{~nm}^{2}$ 4-MBA molecule is about $0.2 \mathrm{~nm}^{2}$, So the total number of Raman reporter molecule sites on the surface of each 30 nm AuNP was 14130 .

Note that the concentration of NTP-modified NPs in the sensing systems was constant.

The concentration of $4-\mathrm{MBA}-\mathrm{AuNP}$ in dimer $=4.32 / 2=2.16 \mathrm{nM}$. The concentration of

4-MBA-AuNP in tetramer equaled to $4.32 / 4=1.08 \mathrm{nM}$.

The equivalent concentration of ligands on NPs in dimer solution $=2.16 \times 14130=$ 30520.8 nM .

The equivalent concentration of ligands on NPs in tetramer solution $=1.08 \times 14130$ $=15260.4 \mathrm{nM}$.


Figure S8. Raman spectrum of $40 \mathrm{mM} 4-\mathrm{MBA}$.
To determine the SERS EF, the Raman signals of freestanding NTP in water were used as a reference, where 0.04 M NTP gave a Raman peak at $1064 \mathrm{~cm}^{-1}$ with the intensity of 607.

EF of AuNP dimer $\quad E F_{4-M B A ~ i n ~ d i m e r s ~}=\frac{I_{4-\text { MBA in dimers }}}{I_{4-M B A}} \times \frac{N_{4-\text { MBA }}}{N_{4-\text { MBA in dimers }}}$
EF of AuNP tetramer $\quad E F_{4-M B A}$ in dimers $=\frac{I_{4-\text { MBA }} \text { in tetramers }}{I_{4-M B A}} \times \frac{N_{4-M B A}}{N_{4-M B A} \text { in tetramers }}$
The EF of NTP in dimers and tetramer was $3.53 \times 10^{3}$ and $2.5 \times 10^{4}$, respectively.

Sequences of Oligonucleotides Used in This Work

Sequence of staple strands used in the assembly of basic rectangular DNA origami (left to right $5^{\prime}-3^{\prime}$ )

1 CAAGCCCAATAGGAAC CCATGTACAAACAGTT 2 AATGCCCCGTAACAGT GCCCGTATCTCCCTCA 3 TGCCTTGACTGCCTAT TTCGGAACAGGGATAG 4 GAGCCGCCCCACCACC GGAACCGCGACGGAAA 5 AACCAGAGACCCTCAG AACCGCCAGGGGTCAG 6 TTATTCATAGGGAAGG TAAATATT CATTCAGT 7 CATAACCCGAGGCATA GTAAGAGC TTTTTAAG 8 ATTGAGGGTAAAGGTG AATTATCAATCACCGG 9 AAAAGTAATATCTTAC CGAAGCCCTTCCAGAG 10 GCAATAGCGCAGATAG CCGAACAATTCAACCG 11 CCTAATTTACGCTAAC GAGCGTCTAATCAATA 12 TCTTACCAGCCAGTTA CAAAATAAATGAAATA 13 ATCGGCTGCGAGCATG TAGAAACCTATCATAT 14 CTAATTTATCTTTCCT TATCATTCATCCTGAA 15 GCGTTATAGAAAAAGC CTGTTTAG AAGGCCGG 16 GCTCATTTTCGCATTA AATTTTTG AGCTTAGA 17 AATTACTACAAATTCT TACCAGTAATCCCATC 18 TTAAGACGTTGAAAAC ATAGCGATAACAGTAC 19 TAGAATCCCTGAGAAG AGTCAATAGGAATCAT 20 CTTTTACACAGATGAA TATACAGTAAACAATT 21 TTTAACGTTCGGGAGA AACAATAATTTTCCCT 22 CGACAACTAAGTATTA GACTTTACAATACCGA

23 GGATTTAGCGTATTAA ATCCTTTGTTTTCAGG

24 ACGAACCAAAACATCG CCATTAAA TGGTGGTT
25 GAACGTGGCGAGAAAG GAAGGGAA CAAACTAT
26 TAGCCCTACCAGCAGA AGATAAAAACATTTGA 27 CGGCCTTGCTGGTAAT ATCCAGAACGAACTGA 28 CTCAGAGCCACCACCC TCATTTTCCTATTATT 29 CTGAAACAGGTAATAA GTTTTAACCCCTCAGA 30 AGTGTACTTGAAAGTA TTAAGAGGCCGCCACC 31 GCCACCACTCTTTTCA TAATCAAACCGTCACC 32 GTTTGCCACCTCAGAG CCGCCACCGATACAGG 33 GACTTGAGAGACAAAA GGGCGACAAGTTACCA 34 AGCGCCAACCATTTGG GAATTAGATTATTAGC 35 GAAGGAAAATAAGAGC AAGAAACAACAGCCAT 36 GCCCAATACCGAGGAA ACGCAATAGGTTTACC

37 ATTATTTAACCCAGCT ACAATTTTCAAGAACG 38 TATTTTGCTCCCAATC CAAATAAGTGAGTTAA 39 GGTATTAAGAACAAGA AAAATAATTAAAGCCA 40 TAAGTCCTACCAAGTA CCGCACTCTTAGTTGC 41 ACGCTCAAAATAAGAA TAAACACCGTGAATTT 42 AGGCGTTACAGTAGGG CTTAATTGACAATAGA 43 ATCAAAATCGTCGCTA TTAATTAACGGATTCG 44 CTGTAAATCATAGGTC TGAGAGACGATAAATA


67 TAACCTCCATATGTGA GTGAATAAACAAAATC 68 AAATCAATGGCTTAGG TTGGGTTACTAAATTT 69 GCGCAGAGATATCAAA ATTATTTGACATTATC 70 AACCTACCGCGAATTA TTCATTTCCAGTACAT 71 ATTTTGCGTCTTTAGG AGCACTAAGCAACAGT 72 CTAAAATAGAACAAAG AAACCACCAGGGTTAG 73 GCCACGCTATACGTGG CACAGACAACGCTCAT 74 GCGTAAGAGAGAGCCA GCAGCAAAAAGGTTAT 75 GGAAATACCTACATTT TGACGCTCACCTGAAA 76 TATCACCGTACTCAGG AGGTTTAGCGGGGTTT 77 TGCTCAGTCAGTCTCT GAATTTACCAGGAGGT 78 GGAAAGCGACCAGGCG GATAAGTGAATAGGTG 79 TGAGGCAGGCGTCAGA CTGTAGCGTAGCAAGG 80 TGCCTTTAGTCAGACG ATTGGCCTGCCAGAAT 81 CCGGAAACACACCACG GAATAAGTAAGACTCC 82 ACGCAAAGGTCACCAA TGAAACCAATCAAGTT 83 TTATTACGGTCAGAGG GTAATTGAATAGCAGC 84 TGAACAAACAGTATGT TAGCAAACTAAAAGAA 85 CTTTACAGTTAGCGAA CCTCCCGACGTAGGAA 86 GAGGCGTTAGAGAATA ACATAAAAGAACACCC 87 TCATTACCCGACAATA AACAACATATTTAGGC 88 CCAGACGAGCGCCCAA TAGCAAGCAAGAACGC

89 AGAGGCATAATTTCAT CTTCTGACTATAACTA 90 TTTTAGTTTTTCGAGC CAGTAATAAATTCTGT

91 TATGTAAACCTTTTTT AATGGAAAAATTACCT 92 TTGAATTATGCTGATG CAAATCCACAAATATA 93 GAGCAAAAACTTCTGA ATAATGGAAGAAGGAG

94 TGGATTATGAAGATGA TGAAACAAAATTTCAT 95 CGGAATTATTGAAAGG AATTGAGGTGAAAAAT 96 ATCAACAGTCATCATA TTCCTGATTGATTGTT 97 CTAAAGCAAGATAGAA CCCTTCTGAATCGTCT 98 GCCAACAGTCACCTTG CTGAACCTGTTGGCAA 99 GAAATGGATTATTTAC ATTGGCAGACATTCTG 100 TTTT TATAAGTA TAGCCCGGCCGTCGAG 101 AGGGTTGA TTTT ATAAATCC TCATTAAATGATATTC 102 ACAAACAA TTTT AATCAGTA GCGACAGATCGATAGC 103 AGCACCGT TTTT TAAAGGTG GCAACATAGTAGAAAA 104 TACATACA TTTT GACGGGAG AATTAACTACAGGGAA 105 GCGCATTA TTTT GCTTATCC GGTATTCTAAATCAGA 106 TATAGAAG TTTT CGACAAAA GGTAAAGTAGAGAATA 107 TAAAGTAC TTTT CGCGAGAA AACTTTTTATCGCAAG 108 ACAAAGAA TTTT ATTAATTA CATTTAACACATCAAG

109 AAAACAAA TTTT TTCATCAA TATAATCCTATCAGAT 110 GATGGCAA TTTT AATCAATA TCTGGTCACAAATATC

111 AAACCCTC TTTT ACCAGTAA TAAAAGGGATTCACCA GTCACACG TTTT

112 CCGAAATCCGAAAATC CTGTTTGAAGCCGGAA 113 CCAGCAGGGGCAAAAT CCCTTATAAAGCCGGC 114 GCATAAAGTTCCACAC AACATACGAAGCGCCA 115 GCTCACAATGTAAAGC CTGGGGTGGGTTTGCC 116 TTCGCCATTGCCGGAA ACCAGGCATTAAATCA 117 GCTTCTGGTCAGGCTG CGCAACTGTGTTATCC 118 GTTAAAATTTTAACCA ATAGGAACCCGGCACC 119 AGACAGTCATTCAAAA GGGTGAGAAGCTATAT 120 AGGTAAAGAAATCACC ATCAATATAATATTTT 121 TTTCATTTGGTCAATA ACCTGTTTATATCGCG 122 TCGCAAATGGGGCGCG AGCTGAAATAATGTGT 123 TTTTAATTGCCCGAAA GACTTCAAAACACTAT 124 AAGAGGAACGAGCTTC AAAGCGAAGATACATT 125 GGAATTACTCGTTTAC CAGACGACAAAAGATT 126 GAATAAGGACGTAACA AAGCTGCTCTAAAACA 127 CCAAATCACTTGCCCT GACGAGAACGCCAAAA 128 CTCATCTTGAGGCAAAAGAATACAGTGAATTT 129 AAACGAAATGACCCCC AGCGATTATTCATTAC 130 CTTAAACATCAGCTTG CTTTCGAGCGTAACAC 131 TCGGTTTAGCTTGATA CCGATAGTCCAACCTA


154 CAATGACACTCCAAAA GGAGCCTTACAACGCC 155 AAAAAAGGACAACCAT CGCCCACGCGGGTAAA 156 TGTAGCATTCCACAGA CAGCCCTCATCTCCAA 157 GTAAAGCACTAAATCG GAACCCTAGTTGTTCC 158 AGTTTGGAGCCCTTCA CCGCCTGGTTGCGCTC 159 AGCTGATTACAAGAGT CCACTATTGAGGTGCC 160 ACTGCCCGCCGAGCTC GAATTCGTTATTACGC 161 CCCGGGTACTTTCCAG TCGGGAAACGGGCAAC 162 CAGCTGGCGGACGACG ACAGTATCGTAGCCAG 163 GTTTGAGGGAAAGGGG GATGTGCTAGAGGATC 164 CTTTCATCCCCAAAAA CAGGAAGACCGGAGAG 165 AGAAAAGCAACATTAA ATGTGAGCATCTGCCA 166 GGTAGCTAGGATAAAA ATTTTTAGTTAACATC 167 CAACGCAATTTTTGAG AGATCTACTGATAATC 168 CAATAAATACAGTTGA TTCCCAATTTAGAGAG 169 TCCATATACATACAGG CAAGGCAACTTTATTT 170 TACCTTTAAGGTCTTT ACCCTGACAAAGAAGT 171 CAAAAATCATTGCTCC TTTTGATAAGTTTCAT 172 TTTGCCAGATCAGTTG AGATTTAGTGGTTTAA 173 AAAGATTCAGGGGGTA ATAGTAAACCATAAAT 174 TTTCAACTATAGGCTG GCTGACCTTGTATCAT 175 CCAGGCGCTTAATCAT TGTGAATTACAGGTAG

176 CGCCTGATGGAAGTTT CCATTAAACATAACCG 177 TTTCATGAAAATTGTG TCGAAATCTGTACAGA 178 ATATATTCTTTTTTCA CGTTGAAAATAGTTAG 179 AATAATAAGGTCGCTG AGGCTTGCAAAGACTT 180 CGTAACGATCTAAAGT TTTGTCGTGAATTGCG 181 ACCCAAATCAAGTTTT TTGGGGTCAAAGAACG 182 TGGACTCCCTTTTCAC CAGTGAGACCTGTCGT 183 TGGTTTTTAACGTCAA AGGGCGAAGAACCATC 184 GCCAGCTGCCTGCAGG TCGACTCTGCAAGGCG 185 CTTGCATGCATTAATG AATCGGCCCGCCAGGG 186 ATTAAGTTCGCATCGT AACCGTGCGAGTAACA 187 TAGATGGGGGGTAACG CCAGGGTTGTGCCAAG 188 ACCCGTCGTCATATGT ACCCCGGTAAAGGCTA 189 CATGTCAAGATTCTCC GTGGGAACCGTTGGTG 190 TCAGGTCACTTTTGCG GGAGAAGCAGAATTAG 191 CTGTAATATTGCCTGA GAGTCTGGAAAACTAG 192 CAAAATTAAAGTACGG TGTCTGGAAGAGGTCA 193 TGCAACTAAGCAATAA AGCCTCAGTTATGACC 194 TTTTTGCGCAGAAAAC GAGAATGAATGTTTAG 195 AAACAGTTGATGGCTT AGAGCTTATTTAAATA 196 ACTGGATAACGGAACA ACATTATTACCTTATG 197 ACGAACTAGCGTCCAA TACTGCGGAATGCTTT

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1 9 8 \text { CGATTTTAGAGGACAG ATGAACGGCGCGACCT}
1 9 9 ~ C T T T G A A A A G A A C T G G ~ C T C A T T A T T T A A T A A A ~
200 GCTCCATGAGAGGCTT TGAGGACTAGGGAGTT
201 ACGGCTACTTACTTAG CCGGAACGCTGACCAA
202 AAAGGCCGAAAGGAAC AACTAAAGCTTTCCAG
203 GAGAATAGCTTTTGCG GGATCGTCGGGTAGCA
204 ACGTTAGTAAATGAAT TTTCTGTAAGCGGAGT
205 TTTT CGATGGCC CACTACGTAAACCGTC
206 TATCAGGG TTTT CGGTTTGC GTATTGGGAACGCGCG
207 GGGAGAGG TTTT TGTAAAAC GACGGCCATTCCCAGT
208 CACGACGT TTTT GTAATGGG ATAGGTCAAAACGGCG
2 0 9 \text { GATTGACC TTTT GATGAACG GTAATCGTAGCAAACA}
210 AGAGAATC TTTT GGTTGTAC CAAAAACAAGCATAAA
2 1 1 ~ G C T A A A T C ~ T T T T ~ C T G T A G C T ~ C A A C A T G T A T T G C T G A ~
2 1 2 ~ A T A T A A T G ~ T T T T ~ C A T T G A A T ~ C C C C C T C A A A T C G T C A ~
2 1 3 ~ T A A A T A T T ~ T T T T ~ G G A A G A A A ~ A A T C T A C G A C C A G T C A ~
2 1 4 \text { GGACGTTG TTTT TCATAAGG GAACCGAAAGGCGCAG}
215 ACGGTCAA TTTT GACAGCAT CGGAACGAACCCTCAG
2 1 6 ~ C A G C G A A A A ~ T T T T ~ A C T T T C A ~ A C A G T T T ~ C T G G G A ~ T T T T G C T ~
AAACTTTT
For assembly of AuNP dimer (left to right \(5^{\prime}-3^{\prime}\) )

AAAAAAAAAAAAAAA TCAATTCTTTTAGTTT GACCATTACCAGACCG AAAAAAAAAAAAAAA GAAGCAAAAAAGCGGA TTGCATCAGATAAAAA AAAAAAAAAAAAAAA CCAAAATATAATGCAG ATACATAAACACCAGA AAAAAAAAAAAAAAA CAACGCAATTTTTGAG AGATCTACTGATAATC AAAAAAAAAAAAAAA TCCATATACATACAGG CAAGGCAACTTTATTT AAAAAAAAAAAAAAA CAAAAATCATTGCTCC TTTTGATAAGTTTCAT AAAAAAAAAAAAAAA AAAGATTCAGGGGGTA ATAGTAAACCATAAAT AAAAAAAAAAAAAAA GAAGGAAAATAAGAGC AAGAAACAACAGCCAT AAAAAAAAAAAAAAA ATTATTTAACCCAGCT ACAATTTTCAAGAACG AAAAAAAAAAAAAAA GGTATTAAGAACAAGA AAAATAATTAAAGCCA AAAAAAAAAAAAAAA ACGCTCAAAATAAGAA TAAACACCGTGAATTT AAAAAAAAAAAAAAA ATCAGAGAAAGAACTG GCATGATTTTATTTTG AAAAAAAAAAAAAAA AGGTTTTGAACGTCAA AAATGAAAGCGCTAAT AAAAAAAAAAAAAAA AATGCAGACCGTTTTT ATTTTCATCTTGCGGG AAAAAAAAAAAAAAA AATGGTTTACAACGCC AACATGTAGTTCAGCT For assembly of AuNP tetramer (left to right \(5^{\prime}-3\) ')

AAAAAAAAAAAAAAA TTTCATGAAAATTGTG TCGAAATCTGTACAGA AAAAAAAAAAAAAAA GCGAAACATGCCACTA CGAAGGCATGCGCCGA AAAAAAAAAAAAAAA AAAGATTCAGGGGGTA ATAGTAAACCATAAAT AAAAAAAAAAAAAAA ACCGTTCTAAATGCAA TGCCTGAGAGGTGGCA AAAAAAAAAAAAAAA CCCGGGTACTTTCCAG TCGGGAAACGGGCAAC AAAAAAAAAAAAAAA GTGAGCTAGTTTCCTG TGTGAAATTTGGGAAG```

