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Supplementary Materials for

Quantizing single-molecule surface-enhanced Raman scattering with DNA origami metamolecules

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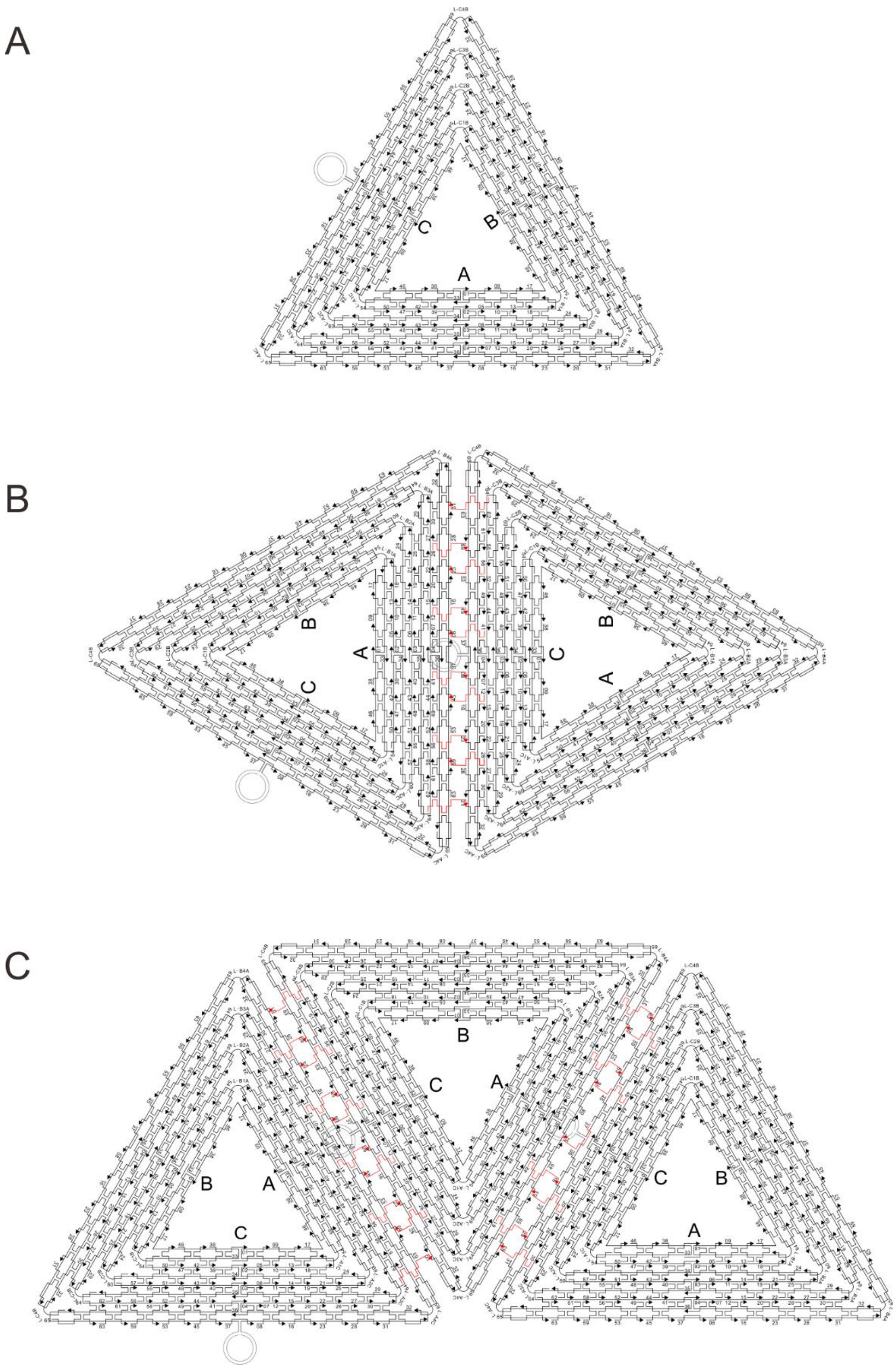

Fig. S1. Schematic drawings of DNA origami template. (A) Single triangular origami. (B) The rhombus-shaped super-origami. (C) The trapezoid-shaped super-origami.

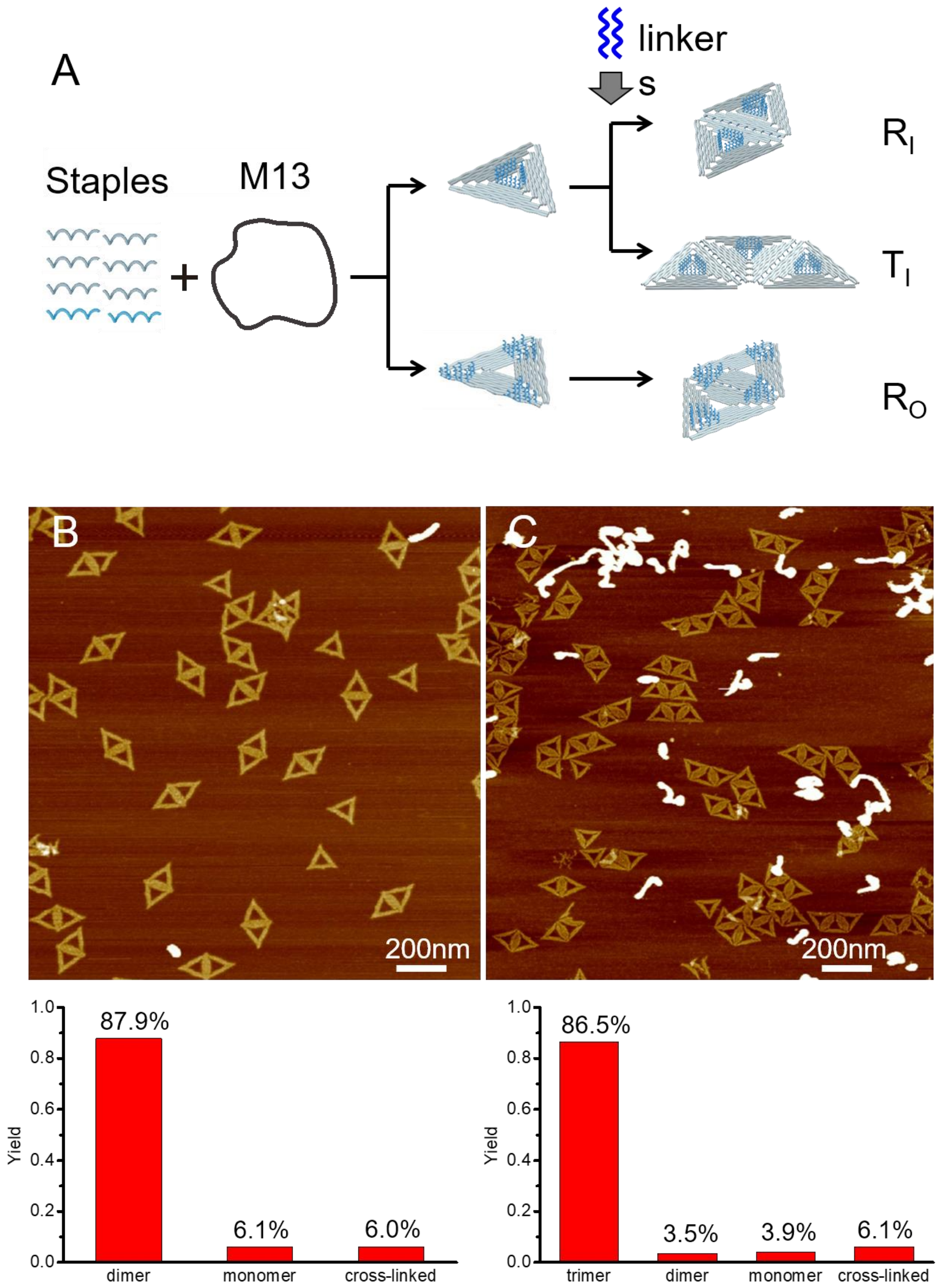

Fig. S2. Super-origami templates. (A) Schematic illustration of the building procedure. Super-origami templates were assembled from two triangular origami units with site-specific anchors. (B) AFM image and the counted yield of the rhombus-shaped super-origami (N=132). (C) AFM image and the counted yield of the trapezoid-shaped super-origami (N=229).

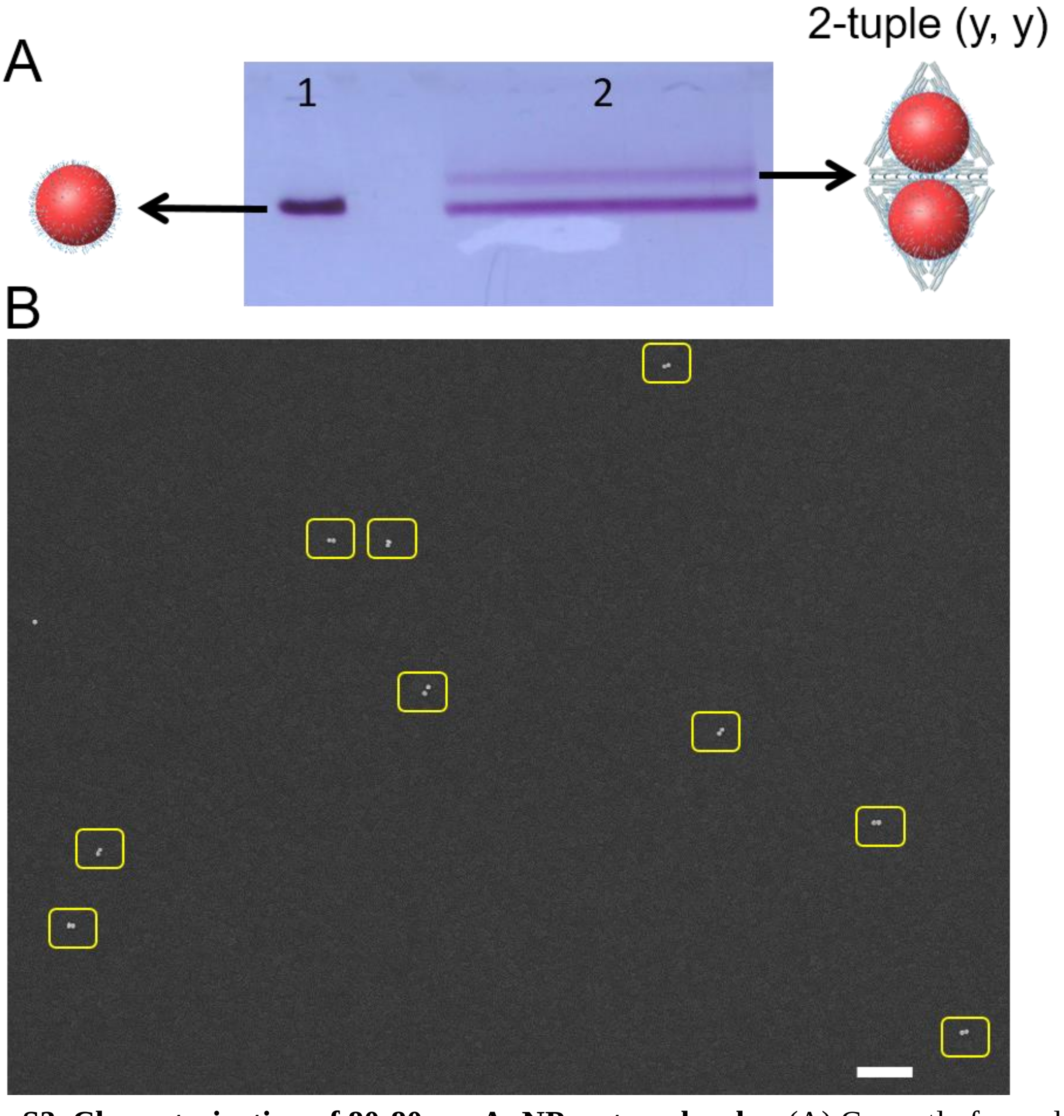

Fig. S3. Characterization of 80-80 nm AuNP metamolecules. (A) Correctly formed homo-dimer metamolecules were separated from free AuNPs and aggregates by gel electrophoresis running on a 0.5% agarose gel. (B) SEM image of the 2-tuple (y, y) metamolecules. Scale bar, 1 µm.

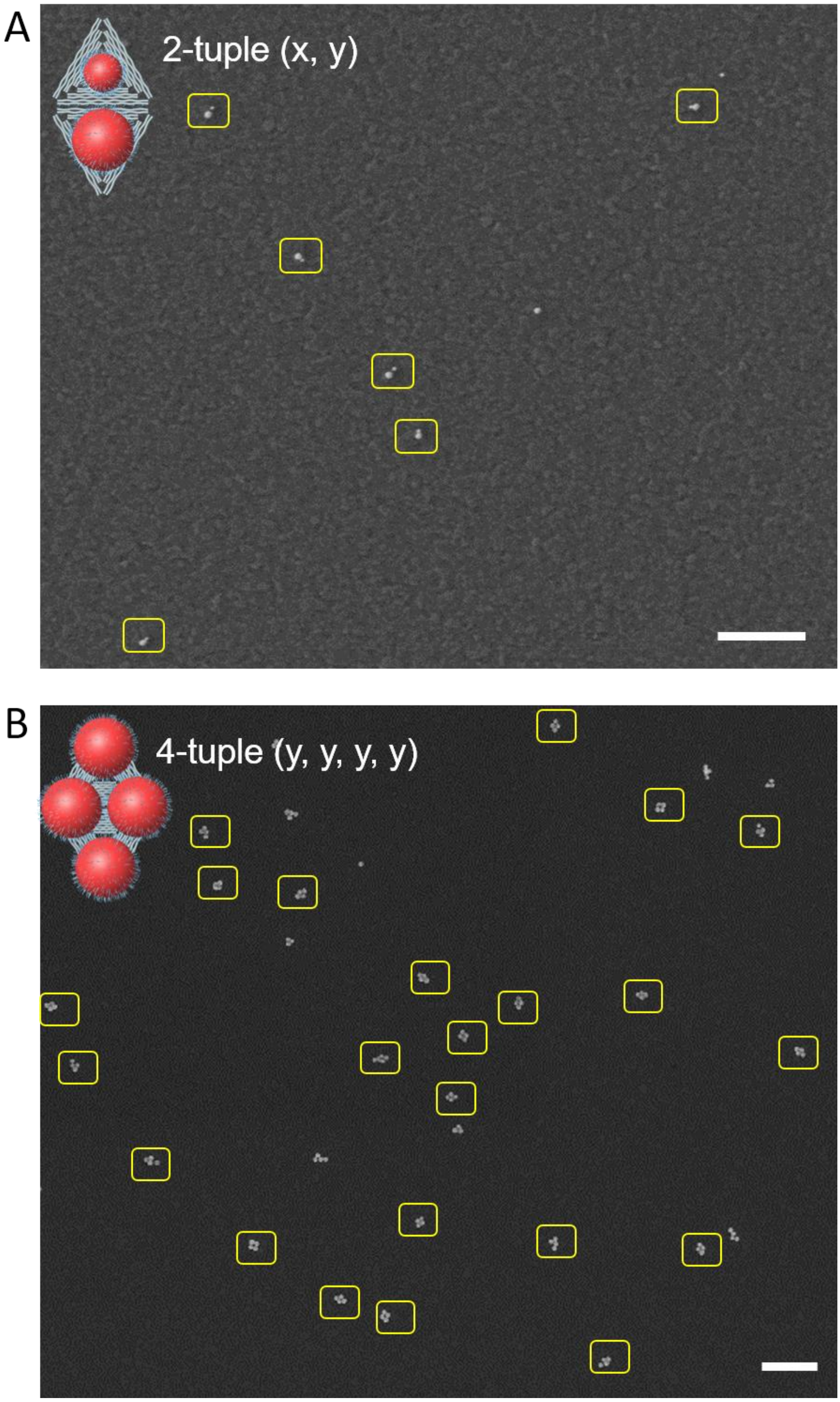

Fig. S4. SEM images of 50-80 nm and 80-80-80-80 nm AuNP metamolecules. (A) SEM image of the 2-tuple (x, y). (B) SEM image of the 4-tuple (y, y, y, y). Scale bars, 1 µm.

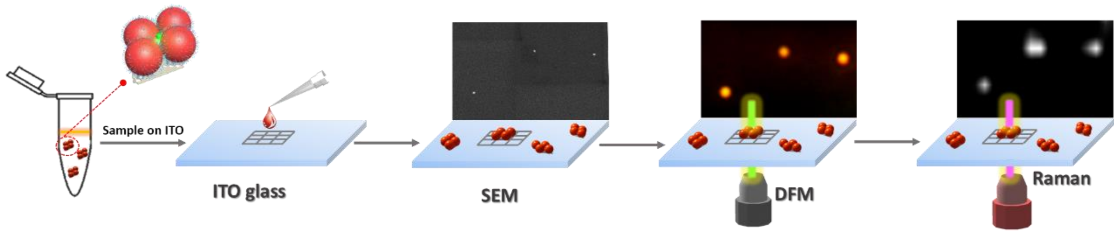

Fig. S5. Schematic representation of SEM-DFM-Raman correlative imaging for plasmonic property investigations. In a typical procedure, a diluted solution of a metamolecule sample was deposited on the ITO glass and characterized with co-localized SEM, DFM and Raman, respectively.

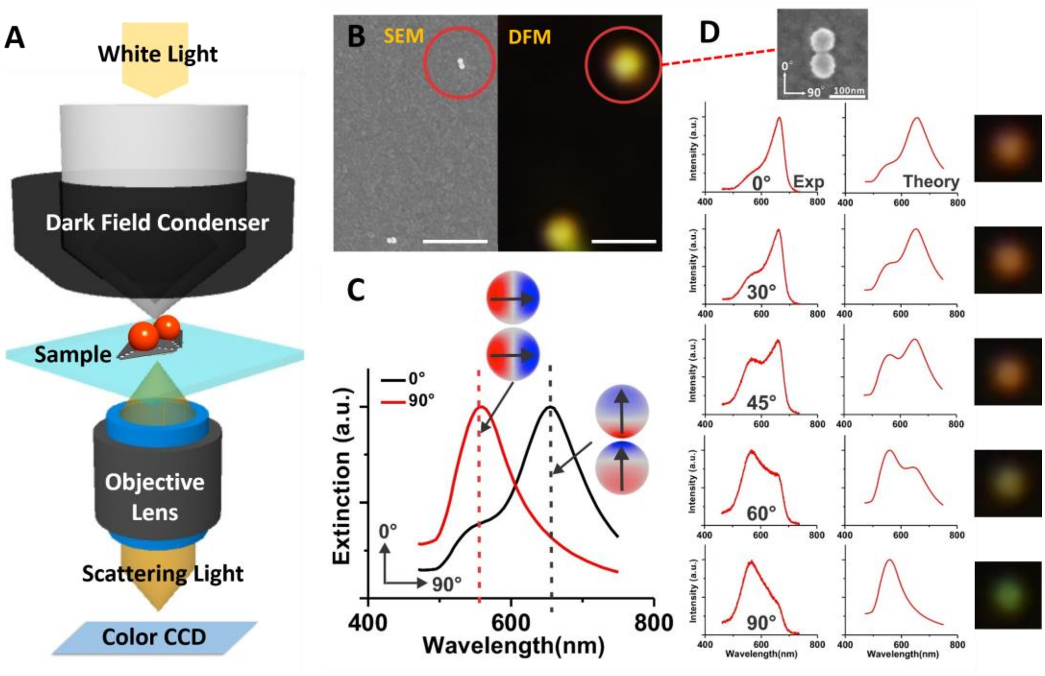

Fig. S6. Characterization of the plasmonic properties of 80-nm homodimer [tuple (*y*, *y*)] using the "DFM-SEM correlative imaging." (A) Experimental DFM setup. (B) SEM image and DFM image at the same position of an 80 nm AuNP dimer. Scale bars, 1 µm. (C) The theoretic extinction spectra and surface charge distribution plot of the 80 nm dimer when the polarization angles of the incident light are 0 and 90° respectively. (D) SEM image and scattering spectra (the experimental and theoretic) of the 80 nm dimer in the red circle in (B) at different polarization angles of incident light. The orientation angles of the incident light relative to the cluster are shown in the SEM image. The scattering spectra were integrated as 20 seconds.

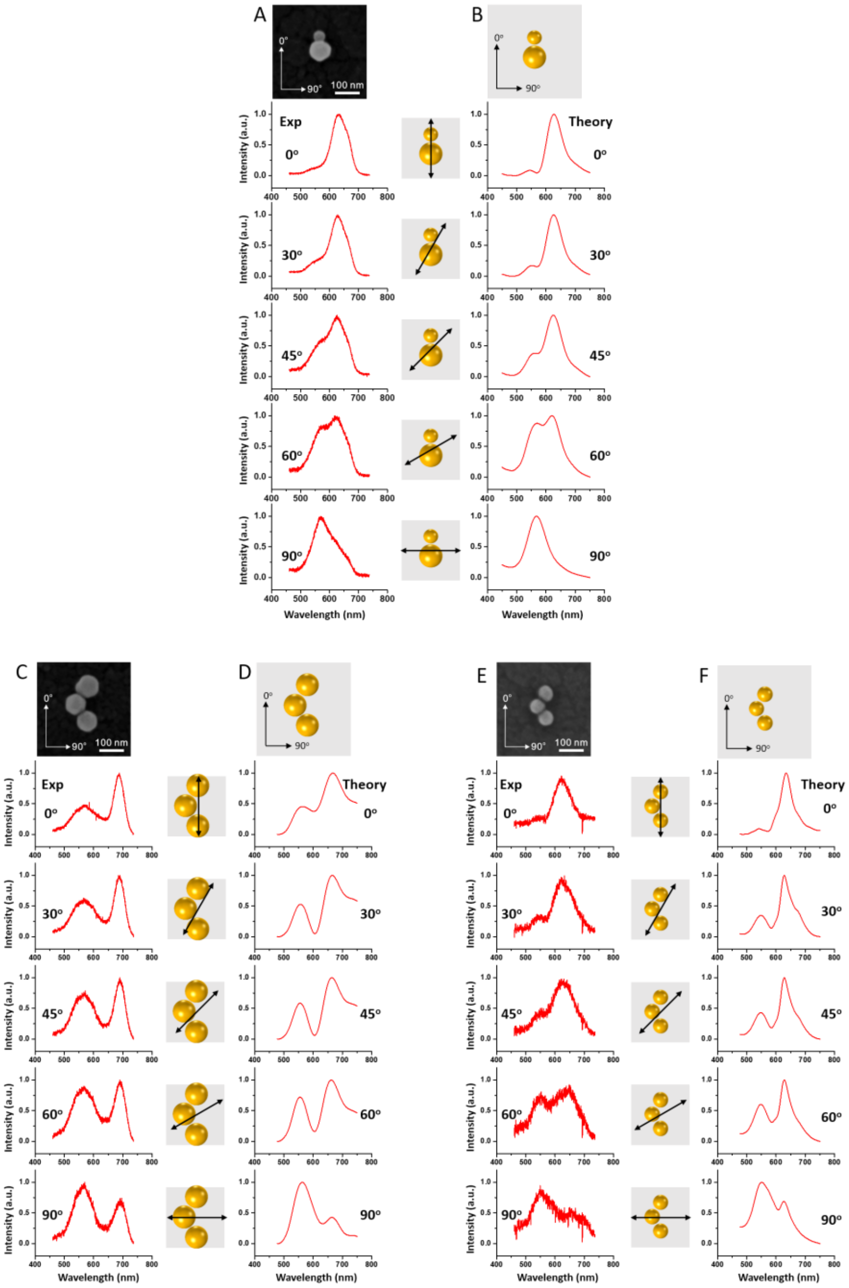

Fig. S7. Characterization of the plasmonic properties of three metamolecules using the "DFM-SEM correlative imaging." (A, B) Experimental and theoretic scattering spectra of a hetero-trimer [2-tuple (x, y)] at different polarization angles of incident light. The orientation angles of the incident light relative to the cluster are shown in the middle column. The scattering spectra were integrated as 20 seconds. (C, D) Experimental and theoretic scattering spectra of homo-trimer [3-tuple (y, y, y)] at different polarization angles of incident light. The orientation angles of the incident light relative to the cluster are shown in the middle column. The scattering spectra were integrated as 20 seconds. (E, F) Experimental and theoretic scattering spectra of homo-trimer [3-tuple (x, x, x)] at different polarization angles of incident light. The orientation angles of the incident light relative to the cluster are shown in the middle column. The scattering spectra were integrated as 30 seconds.

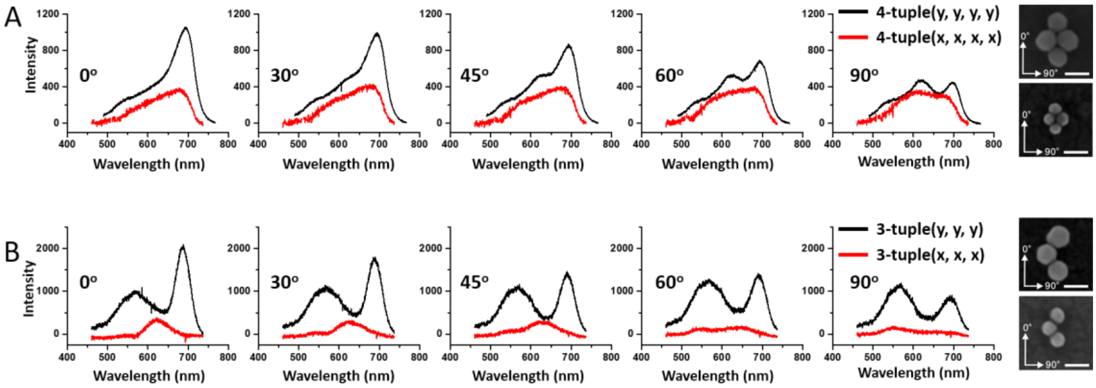

Fig. S8. The absolute scattering spectra of two homotetramers and two homotrimers. (A) Experimental scattering spectra at different polarization angles of incident light and SEM images of two homo-tetramers [4-tuple (x, x, x, x) and 4-tuple (y, y, y, y)]. The scattering spectra were integrated as 10 seconds for 4-tuple (y, y, y, y) and 30 seconds for 4-tuple (x, x, x, x), respectively. (B) Experimental scattering spectra at different polarization angles of incident light and SEM images of two homo-trimers [tuple (x, x, x) and tuple (y, y, y)]. The scattering spectra were integrated as 20 seconds for 3-tuple (y, y, y) and 30 seconds for 3-tuple (x, x, x), respectively. Scale bars, 100 nm.

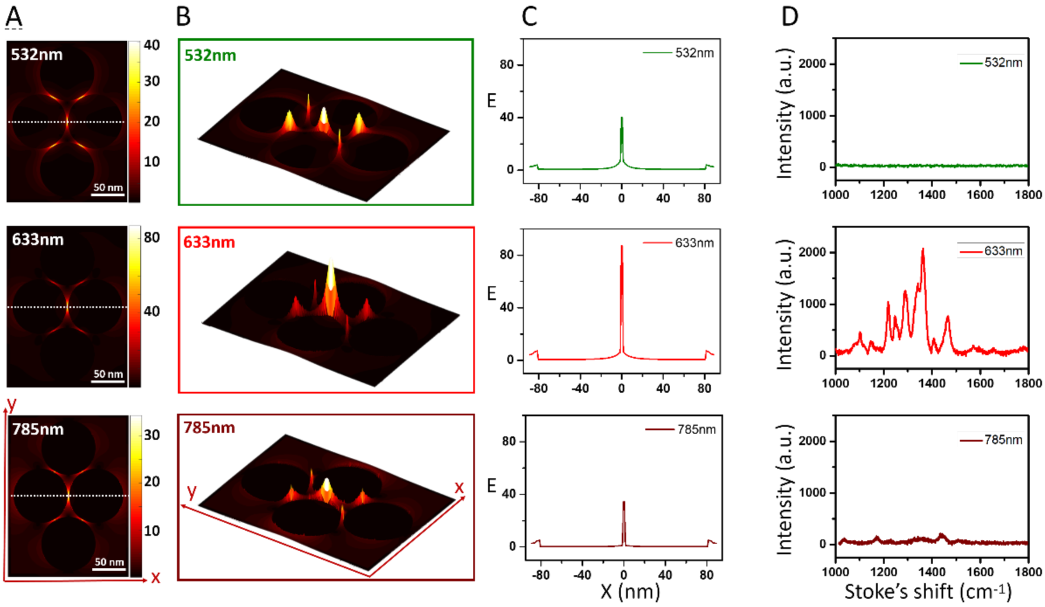

Fig. S9. FDTD calculations of the electromagnetic field (E) at mid-height of the tetrameric metamolecules. (A) FDTD calculated E enhancement at a wavelength of 532-nm, 633-nm and 785-nm. (B) 3D surface plot of the peak intensity variation across the tetrameric metamolecule at a wavelength of 532-nm, 633-nm and 785-nm. (C) The line electromagnetic field distribution profile along the center-horizontal line at an incident wavelength of 532-nm, 633-nm and 785-nm. (D) SERS spectra of three different incident wavelengths (green line, 532-nm; red line, 633-nm; wine red line, 785-nm) for three different SYBR Green I-modified tetrameric metamolecules in air. The incident light was polarized along the tetrameric metamolecule short axis (x-direction). The strongest enhancement was observed in the central gap region at 633-nm, and the calculated EF values of Raman enhancement was 5.7 x 10^7. All spectra were taken with a 633-nm excitation laser, 10-s exposure for a single spectrum.

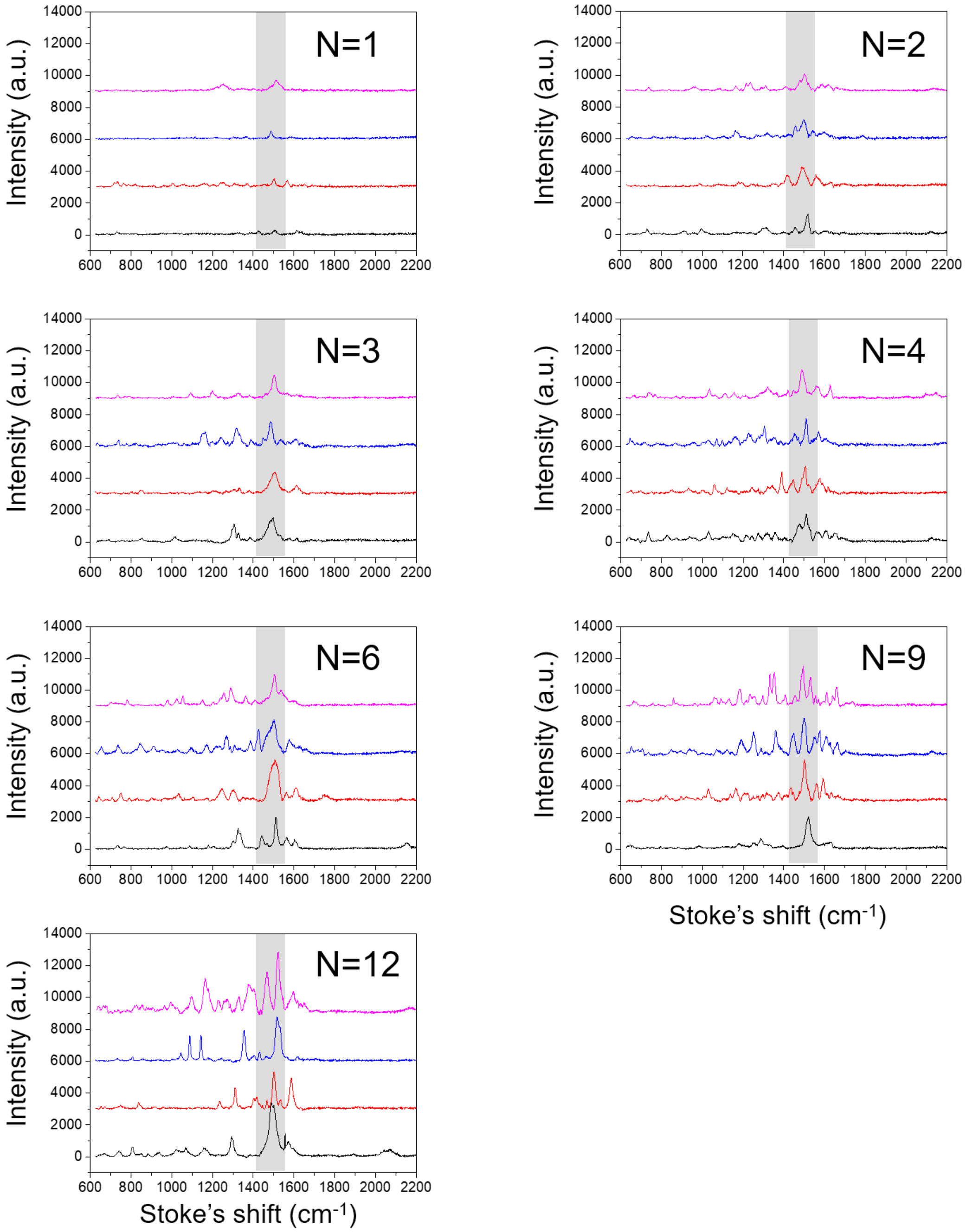

Fig. S10. SERS spectra taken from individual tetrameric metamolecules with different numbers of ROX. The corresponding Raman signal of ROX dye was obtained at a mapping step of 1.5 µm × 1.5 µm. All spectra were taken with a 633-nm excitation laser, 10-s exposure for a single spectrum.

Supplementary Appendix

Sequences of staple strands containing sticky ends

There are totally 20 staple strands are modified with sticky ends to organize two triangular origami into a rhombus-shaped super-origami. Sequence of the staple strands of the triangular A (the side A of one triangular origami was modified) and triangular C (the side C of the other triangular origami was modified) (left to right: 5'-3'):

A64h, ACGACAATAAATCCCGACTTGCGGGAGATCCTGAATCTTACCAGCCACCGA
A59h, CAAAATAATAGAAGGCTTATCCGGTTATCAAC
A56h, ACAAGAAAGCAAGCAAATCAGATAACAGCCATATTATTTATTAAAGGG
A45h, AAATGAAAAGCAAGCCGTTTTTATGAAACCAA
A41h, TTTCCTTAGCACTCATCGAGAACAATAGCAGCCTTTACAGTTGACGAG
A08h, AATTAACTCGGAATAAGTTTATTTCCAGCGCC
A12h, CATTCAACAAACGCAAAGACACCAGAACACCCTGAACAAATAGAGCTT
A23h, ACAAGAATGTTAGCAAACGTAGAAAATTATTC
A26h, CACCGTCACCTTATTACGCAGTATTGAGTTAAGCCCAATACAAGTTTT
A31h, CGAAGCCCAAACGCAATAATAACGAAAATCACCAG
C31h, GTAAAAGAACATCACTTGCCTGAGCGCCATTAAAA
C26h, CTATTAGTATATCCAGAACAATATCAGGAACGGTACGCCAGCCAGTTA
C23h, ATTTTAGATACCGCCAGCCATTGCGGCACAGA
C12h, AGAGATAGTTTGACGCTCAATCGTACGTGCTTTCCTCGTTAACGTCAA
C08h, CACGTATACTGAAATGGATTATTTAATAAAAG
C41h, TTTCACCAGCCTGGCCCTGAGAGAAAGCCGGCGAACGTGGGACGGGAG
C45h, GACGGGGAGTTGCAGCAAGCGGTCATTGGGCG
C56h, TTAATGAAGTTTGATGGTGGTTCCGAGGTGCCGTAAAGCAGATAACCC
C59h, TTGGGGTCGAAATCGGCAAAATCCGGGAAACC
C64h, AACTCACATTATTGAGTGTTGTTCCAGAAACCGTCTATCAGGGTATCTTAC

There are totally 38 staple strands are modified with sticky ends to organize three triangular origami into a trapezoid-shaped super-origami. The three triangle origami are named as **Left** (the side A of triangular origami was modified), **Middle** (the side C and the side A of triangular origami were modified) and **Right** (the side C of triangular origami was modified). The sequence of modified staple strands to link **Left** (the side A) and **Middle** (the side C) are the same as the sequence of the rhombus-shaped super-origami. Sequence of the staple strands to link the **Middle** (the side A) and **Right** (the side C) are as follows (left to right: 5'-3'):

A63h, GAGCGTCTGGCGTTTTAGCGAACCCAACATGT
A61h, GCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTTGAATCCTG
A53h, CAAATAAGATTACCGCGCCCAATAAATAATAT

A49h, AGCATGTATTTCATCGTAGGAATCAAACGATTTTTTGTTTAGAATCAG
A37h, ACATAAAAACAGGGAAGCGCATTA
A16h, GTAATTGATGGCAACATATAAAAGCGATTGAG
A20h, TTGACGGAAATACATACATAAAGGGCGCTAATATCAGAGACTAAATCG
A28h, AAGAAACATGGCATGATTAAGACTCCGACTTG
A30h,TATCTTACCGAAGCCCAAACGCAATAATAACGAAAATCACCAGCGATGGCC
C30h, TAAAACATTAGAAGAACTCAAACTTTTTATAATCAGTGAGACGCTAAC
C28h, AGAAGTGTATCGGCCTTGCTGGTACTTTAATG
C20h, GAATACGTAACAGGAAAAACGCTCCTAAACAGGAGGCCGATCCCAATC
C16h, AGCGGGAGATGGAAATACCTACATAACCCTTC
C37h, CGAGAAAGGAAGGGAAGCGTACTATGGTTGCTAGAGAATA
C49h, GTTTGCGTCACGCTGGTTTGCCCCAAGGGAGCCCCCGATTGTCAGAGG
C53h, GAACCCTAAGCAGGCGAAAATCCTTCGGCCAA
C61h, TTCCAGTCCTTATAAATCAAAAGAGAACCATCACCCAAATATAAGAGC
C63h, CACTACGTATAGCCCGAGATAGGGATTGCGTT

There are two groups of "anchoring strands" that are modified with sticky ends to organize 2-tuple metamolecules and 3-tuple metamolecules (Group 1 and Group 2). Each group contains 18 "anchoring strands" on the inner of the rhombus- (R_I) and trapezoid-shaped (T_I) super-origami.

For dimer (x, x), triangular A: group 1; triangular C: group 1
For dimer (x, y), triangular A: group 1; triangular C: group 2
For dimer (y, x), triangular A: group 2; triangular C: group 1
For dimer (y, y), triangular A: group 2; triangular C: group 2
For trimer (x, x, x), Left: group 1; Middle: group 1; Right: group 1
For trimer (x, y, x), Left: group 1; Middle: group 2; Right: group 1
For trimer (x, x, y), Left: group 1; Middle: group 1; Right: group 2
For trimer (y, x, x), Left: group 2; Middle: group 1; Right: group 1
For trimer (x, y, y), Left: group 1; Middle: group 2; Right: group 2
For trimer (y, x, y), Left: group 2; Middle: group 1; Right: group 2
For trimer (y, y, x), Left: group 2; Middle: group 2; Right: group 1
For trimer (y, y, y), Left: group 2; Middle: group 2; Right: group 2

Sequence of the staple strands (left to right: 5'-3'):

Group 1:

A01-a, AGACTCTAATGCAGTCACCAACGCTTTT
CGGGGTTTCCTCAAGAGAAGGATTTTGAATTA
A09-a, AGACTCTAATGCAGTCACCAACGCTTTT
GATAAGTGCCGTCGAGCTGAAACATGAAAGTATACAGGAG
A17-a, AGACTCTAATGCAGTCACCAACGCTTTTT
AGCCCGGAATAGGTGAATGCCCCCTGCCTATGGTCAGTG
A33-a, AGACTCTAATGCAGTCACCAACGCTTTT
CCTTTTTTCATTTAACAATTTCATAGGATTAG
A38-a, AGACTCTAATGCAGTCACCAACGCTTTT

AAAACAAAATTAATTAAATGGAAACAGTACATTAGTGAAT
A46-a, AGACTCTAATGCAGTCACCAACGCTTTT
GAGCAAAAGAAGATGAGTGAATAACCTTGCTTATAGCTTA
B01-a, AGACTCTAATGCAGTCACCAACGCTTTTT
CATATGTGTAATCGTAAAACTAGTCATTTTC
B09-a, AGACTCTAATGCAGTCACCAACGCTTTT
AGAAAAGCCCCAAAAAGAGTCTGGAGCAAACAATCACCAT
B17-a, AGACTCTAATGCAGTCACCAACGCTTTT
GCAAATATTTAAATTGAGATCTACAAAGGCTACTGATAAA
B33-a, AGACTCTAATGCAGTCACCAACGCTTTT
AGGGATAGCTCAGAGCCACCACCCCATGTCAA
B38-a, AGACTCTAATGCAGTCACCAACGCTTTT
CCTCAGAACCGCCACCCAAGCCCAATAGGAACGTAAATGA
B46-a, AGACTCTAATGCAGTCACCAACGCTTTT
AGGTTTAGTACCGCCATGAGTTTCGTCACCAGGATCTAAA
C01-a, AGACTCTAATGCAGTCACCAACGCTTTT
TCGGGAGATATACAGTAACAGTACAAATAATT
C09-a, AGACTCTAATGCAGTCACCAACGCTTTT
CCTGATTGCTTTGAATTGCGTAGATTTTCAGGCATCAATA
C17-a, AGACTCTAATGCAGTCACCAACGCTTTT
GCGCAGAGGCGAATTAATTATTTGCACGTAAATTCTGAAT
C33-a, AGACTCTAATGCAGTCACCAACGCTTTT
CGCGTCTGATAGGAACGCCATCAACTTTTACA
C38-a, AGACTCTAATGCAGTCACCAACGCTTTT
GCTCATTTTTTAACCAGCCTTCCTGTAGCCAGGCATCTGC
C46-a, AGACTCTAATGCAGTCACCAACGCTTTT
GTTAAAATTCGCATTAATGTGAGCGAGTAACACACGTTGG

Group 2:
A01-b, AATAATAATAATAATAATAATAAT TTTT
CGGGGTTTCCTCAAGAGAAGGATTTTGAATTA
A09-b, AATAATAATAATAATAATAATAAT TTTT
GATAAGTGCCGTCGAGCTGAAACATGAAAGTATACAGGAG
A17-b, AATAATAATAATAATAATAATAAT TTTTT
AGCCCGGAATAGGTGAATGCCCCCTGCCTATGGTCAGTG
A33-b, AATAATAATAATAATAATAATAAT TTTT
CCTTTTTTCATTTAACAATTTCATAGGATTAG
A38-b, AATAATAATAATAATAATAATAAT TTTT
AAAACAAAATTAATTAAATGGAAACAGTACATTAGTGAAT
A46-b, AATAATAATAATAATAATAATAAT TTTT
GAGCAAAAGAAGATGAGTGAATAACCTTGCTTATAGCTTA
B01-b, AATAATAATAATAATAATAATAAT TTTT
CATATGTGTAATCGTAAAACTAGTCATTTTC
B09-b, AATAATAATAATAATAATAATAAT TTTT

AGAAAAGCCCCAAAAAGAGTCTGGAGCAAACAATCACCAT
B17-b, AATAATAATAATAATAATAATAAT TTTT
GCAAATATTTAAATTGAGATCTACAAAGGCTACTGATAAA
B33-b, AATAATAATAATAATAATAATAAT TTTT
AGGGATAGCTCAGAGCCACCACCCCATGTCAA
B38-b, AATAATAATAATAATAATAATAAT TTTT
CCTCAGAACCGCCACCCAAGCCCAATAGGAACGTAAATGA
B46-b, AATAATAATAATAATAATAATAAT TTTT
AGGTTTAGTACCGCCATGAGTTTCGTCACCAGGATCTAAA
C01-b, AATAATAATAATAATAATAATAAT TTTT
TCGGGAGATATACAGTAACAGTACAAATAATT
C09-b, AATAATAATAATAATAATAATAAT TTTT
CCTGATTGCTTTGAATTGCGTAGATTTTCAGGCATCAATA
C17-b, AATAATAATAATAATAATAATAAT TTTT
GCGCAGAGGCGAATTAATTATTTGCACGTAAATTCTGAAT
C33-b, AATAATAATAATAATAATAATAAT TTTT
CGCGTCTGATAGGAACGCCATCAACTTTTACA
C38-b, AATAATAATAATAATAATAATAAT TTTT
GCTCATTTTTTAACCAGCCTTCCTGTAGCCAGGCATCTGC
C46-b, AATAATAATAATAATAATAATAAT TTTT
GTTAAAATTCGCATTAATGTGAGCGAGTAACACACGTTGG

The four corners of a rhombus-shaped super-origami (R_O) are modified with sticky ends to organize 4-tuple metamolecules. Each corner contains 12 “anchoring strands”. The following staples are modified on each corner (clockwise).

Corner 1: B14, B21, B22, B24, B27, B29 (triangular A)
C50, C51, C52, C57, C58, C62 (triangular A)

Corner 2: A14, A21, A22, A24, A27, A29 (triangular A)
C50, C51, C52, C57, C58, C62 (triangular C)

Corner 3: B50, B51, B52, B57, B58, B62 (triangular C)
A14, A21, A22, A24, A27, A29 (triangular C)

Corner 4: A50, A51, A52, A57, A58, A62 (triangular A)
C14, C21, C22, C24, C27, C29 (triangular C)

There are two kinds of modifications of these staples.

Modification 1: the staples were extended with a sequence of AGACTCTAATGCAGTCACCAACGCTTTT on the 5’;

Modification 2: the staples were extended with a sequence of AATAATAATAATAATAATAATAATTTTT on the 5'

For 4-tuple metamolecules, the specific sequences are as follows:

4-tuple	Corner 1	Corner 2	Corner 3	Corner 4
(x, x, x, x)	Modification 1	Modification 1	Modification 1	Modification 1
(y, x, x, x)	Modification 2	Modification 1	Modification 1	Modification 1
(x, y, x, x)	Modification 1	Modification 2	Modification 1	Modification 1
(x, x, y, x)	Modification 1	Modification 1	Modification 2	Modification 1
(x, x, x, y)	Modification 1	Modification 1	Modification 1	Modification 2
(x, x, y, y)	Modification 1	Modification 1	Modification 2	Modification 2
(x, y, x, y)	Modification 1	Modification 2	Modification 1	Modification 2
(x, y, y, x)	Modification 1	Modification 2	Modification 2	Modification 1
(y, y, x, x)	Modification 2	Modification 2	Modification 1	Modification 1
(y, x, y, x)	Modification 2	Modification 1	Modification 2	Modification 1
(y, x, x, y)	Modification 2	Modification 1	Modification 1	Modification 2
(x, y, y, y)	Modification 1	Modification 2	Modification 2	Modification 2
(y, x, y, y)	Modification 2	Modification 1	Modification 2	Modification 2
(y, y, x, y)	Modification 2	Modification 2	Modification 1	Modification 2
(y, y, y, x)	Modification 2	Modification 2	Modification 2	Modification 1
(y, y, y, y)	Modification 2	Modification 2	Modification 2	Modification 2

There are totally 6 staple strands are modified with sticky ends to capture ROX-DNA. The staple sequences were broken to ensure ROX and AuNPs are on the same side. The sequence (S3-A-37m) was used to do the single molecular SERS experiment. Sequence of the staple strands (left to right: 5'-3'):

Triangular A:
S3-A-35m,
CGTTGGGGGCACTATTTCAAGCCACGATCGTTTTTATACAAAGCCATCTT
A-35n, AGTATAAAATATGCGT
S3-A-36m,
CGTTGGGGGCACTATTTCAAGCCACGATCGTTTTAGAACGGGAAATTCAT
A-36n, CAAGTACCTCATTCCA

S3-A-37m,
CGTTGGGGGCACTATTTCAAGCCACGATCGTTTTAGAGAATAACATAAAAA
CAGGGAA
A-37n, AGAGAATA

Triangular C:
S3-C-35m，
CGTTGGGGGCACTATTTCAAGCCACGATCGTTTTCATGCCTGGTCAGTTG
C-35n， CTCTAGAGCAAGCTTG
S3-C-36m，
CGTTGGGGGCACTATTTCAAGCCACGATCGTTTTGGCAACAGCAGTCACA
C-36n, CCTTCACCGTGAGACG
S3-C-37m，
CGTTGGGGGCACTATTTCAAGCCACGATCGTTTTGAAGGGAAGCGTACTAT
GGTTGCT
C-37n, CGAGAAAG

Sequences of ROX modified strands. The strand S1-ROX was modified with ROX on the 5', the strand S5-ROX was modified with ROX on the 3', the other two strands were used to construct the 120bp long double helix structure.
S1-ROX, ROX-TTTTCGGGTTGATCCGTCAACACGCCGCGC
S5-ROX,
CGTTGCGTGTTCTCATAAATTAAGAACACGCGGCGCGGCGTGTTGACGGAT
CAACCCG-ROX
S1, TTTTCGGGTTGATCCGTCAACACGCCGCGC
S2, CGCGTGTTCTTAATTTATGAGAACACGCAACGGCGCGCCGGGACGAT
GAACGCGGCGCCG
S4, CGATCGTGGCTTGAAATAGTGCCCCCAACGCGGCGCCGCGTTCATCG
TCCCGGCGCGC
S5, CGTTGCGTGTTCTCATAAATTAAGAACACGCGGCGCGGCGTGTTGAC
GGATCAACCCG

Sequences of thiolated strands that covered the AuNPs. The thiolated strands are modified with monothiol on the 3'.
Corner, ATTATTATTATTATTATTATTATTTTTT-SH
S5b, GCGTTGGTGACTGCATTAGAGTCTTTTT-SH