Electronic Supplementary Material (ESI) for Molecular Systems Design & Engineering.

Supporting Information

Enabling spectral barcoding of SERS nanotags using gold nanostar

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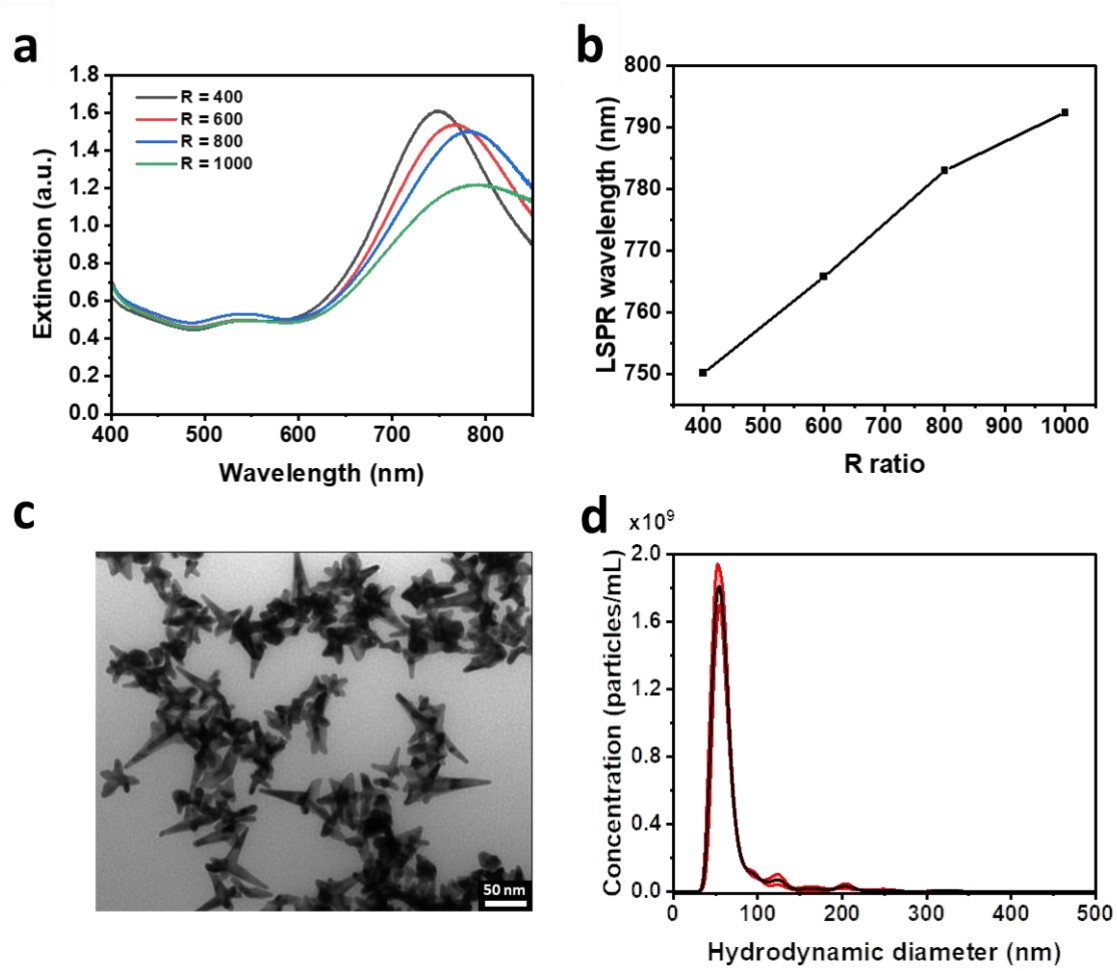


Figure S1. (a) UV-Vis absorption spectra of as-synthesized AuNSs with various molar ratio of HEPES to $HAuCl_4$ (R); (b) LSPR peak wavelength of the synthesized AuNS as function of R value; (c) TEM image of AuNS synthesized at the R = 800 as the LSPR wavelength of this solution was nearest to the laser excitation wavelength of 785 nm (at magnification 130,000 and applied voltage of 100 kV); (d) NTA of three replicates of AuNSs synthesized at R = 800 indicating the average hydrodynamic diameter of each AuNS at 53.5 ± 2.3 nm and the average concentration of AuNSs at $(4.99 \pm 0.06)\times10^{10}$ particles/mL.

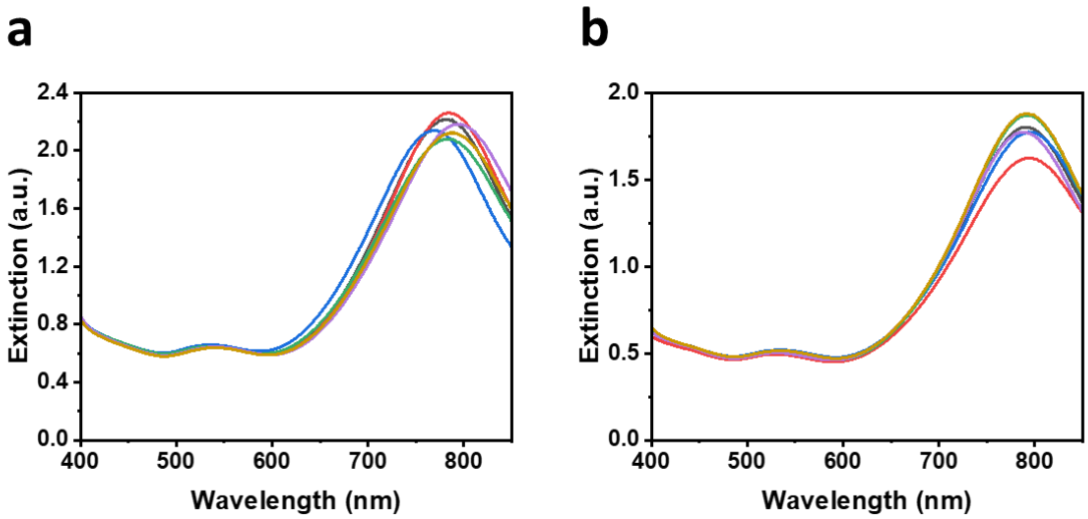


Figure S2. UV-Vis spectra of synthesized AuNSs in different volumes of batches. Reproducibility of synthesized AuNSs as changing the batch size from 10 mL to 20 mL with six vials of AuNS solution at each size. LSPR wavelengths of 10 mL batches and 20 mL batches were in range of 775 – 795 nm (a) and 788 – 794 nm (b).

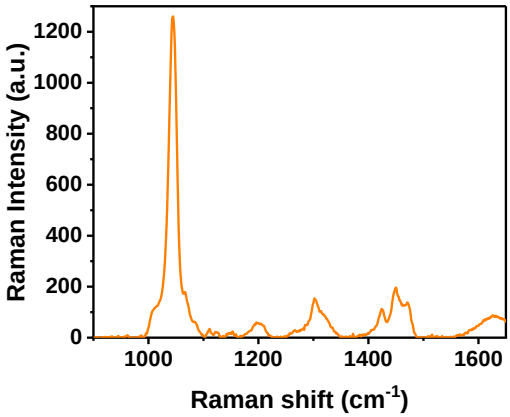
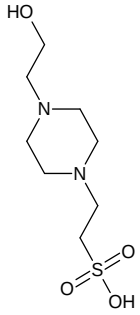


Figure S3. Raman spectrum of 0.2 M of HEPES solution using laser excitation of 785 nm.

Table S1. Band assignments to the Raman fingerprint of HEPES[1-3]

Molecular structure	Raman shift (cm^{-1})	Assignment
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid 	1045	SO_3^- symmetric stretch
	1196	C-N stretch
	1302	CH_2 twist
	1424	CH_2 deformation
	1450	CH_2 scissor
	1625	H-O-H bend

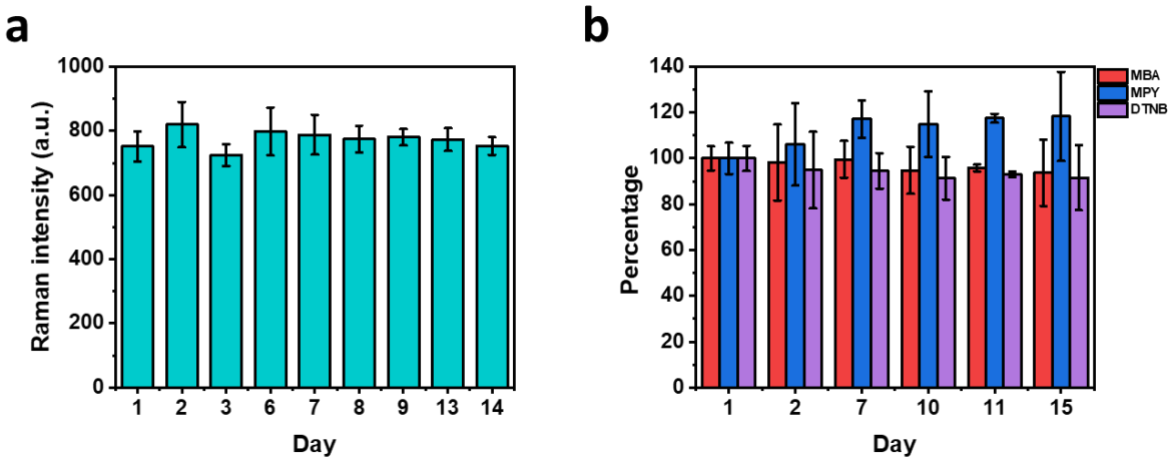


Figure S4. Stability of AuNS@TFMBA (a) and a mixture of three SERS nanotags (b) (AuNS@MBA, AuNS@MPY, and AuNS@DTNB) over two weeks. Raman intensity of each replicate was normalized by its extinction peak intensity then compared to the value on the first day to calculate the percentage. (Error bar stands for three replicates)

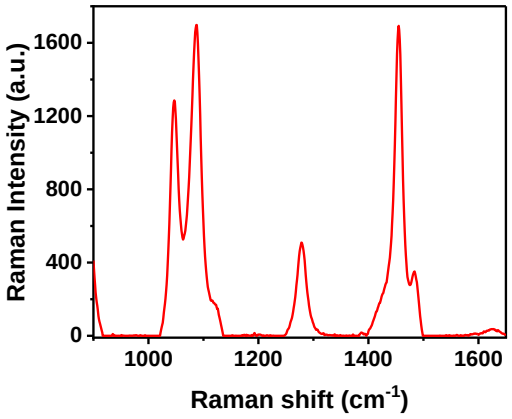


Figure S5. Raman spectrum of 1 M of TFMBA in ethanol**.**

SI-1. Enhancement factor estimation of AuNSs

The diameter (d) and the pathlength (h) of the laser spot in the liquid: d = 40 µm, h = 10 mm.

$$V_{\text{laser spot}} = \pi(\frac{d}{2})^2 \cdot h = \pi(20\ \mu m)^2 \cdot 10\ mm = 1.26 \times 10^{-5}\ cm^3$$

For SERS

As can be seen in ***Figure 3b***, the SERS intensity of AuNS@TFMBA increases with increasing the concentration of TFMBA, where it reaches a platue at concentration about 0.9 mM. It means that 5 µl of TFMBA 0.9 mM could be enough for coating 1 mL of AuNS dispersion.

Thus, number of TFMBA molecules in 1 mL AuNSs solution:

$$N_{TFMBA}\ (AuNS@TFMBA) = (0.9\ mM \times 5\ \mu L) \times N_A$$
$$= \left(0.9 \times 10^{-3}\frac{mol}{L}\right) \times (5 \times 10^{-6}\ L) \times \left(6.02 \times 10^{23}\frac{molecules}{mol}\right)$$
$$= 2.71 \times 10^{15}\ molecules$$

Number of TFMBA molecules in laser spot:

$$N_{SERS}(AuNS@TFMBA) = V_{laser\ spot} \cdot \frac{N_{TFMBA}\ (AuNS@TFMBA)}{1\ mL}$$
$$= 1.26 \times 10^{-5}\ cm^3 \cdot \frac{2.71 \times 10^{15}\ molecules}{1\ mL} = 3.41 \times 10^{10}\ molecules$$

I_{SERS} (AuNS@TFMBA) = 635.31

For Raman

Concentration of TFMBA for reference: 1 M = 1 mol/L

Number of TFMBA molecules in 1L: N'_{TFMBA} =6.02 × 10^{23} molecules

Thus, number of TFMBA in laser spot:

$$N_{Raman} = V_{laser\ spot} \cdot \frac{N_{TFMBA}}{1\ L} = 1.26 \times 10^{-5}\ cm^3 \cdot \frac{6.02 \times 10^{23}\ molecules}{1\ L}$$
$$= 1.26 \times 10^{-5}\ cm^3 \cdot \frac{6.02 \times 10^{23}\ molecules}{1000\ \ cm^3} = 7.59 \times 10^{15}\ molecules$$

I_{Raman} = 5863.85

Overall, EF of AuNS after conjugation with TFMBA can be calculated as below:

$$EF(AuNS) = \frac{I_{SERS}\ (AuNS@TFMBA)}{I_{Raman}} \cdot \frac{N_{Raman}}{N_{SERS}(AuNS@TFMBA)}$$
$$= \frac{635.31}{5863.85} \cdot \frac{7.59 \times 10^{15}\ molecules}{3.41 \times 10^{10}\ \ molecules} = 2.41 \times 10^4.$$

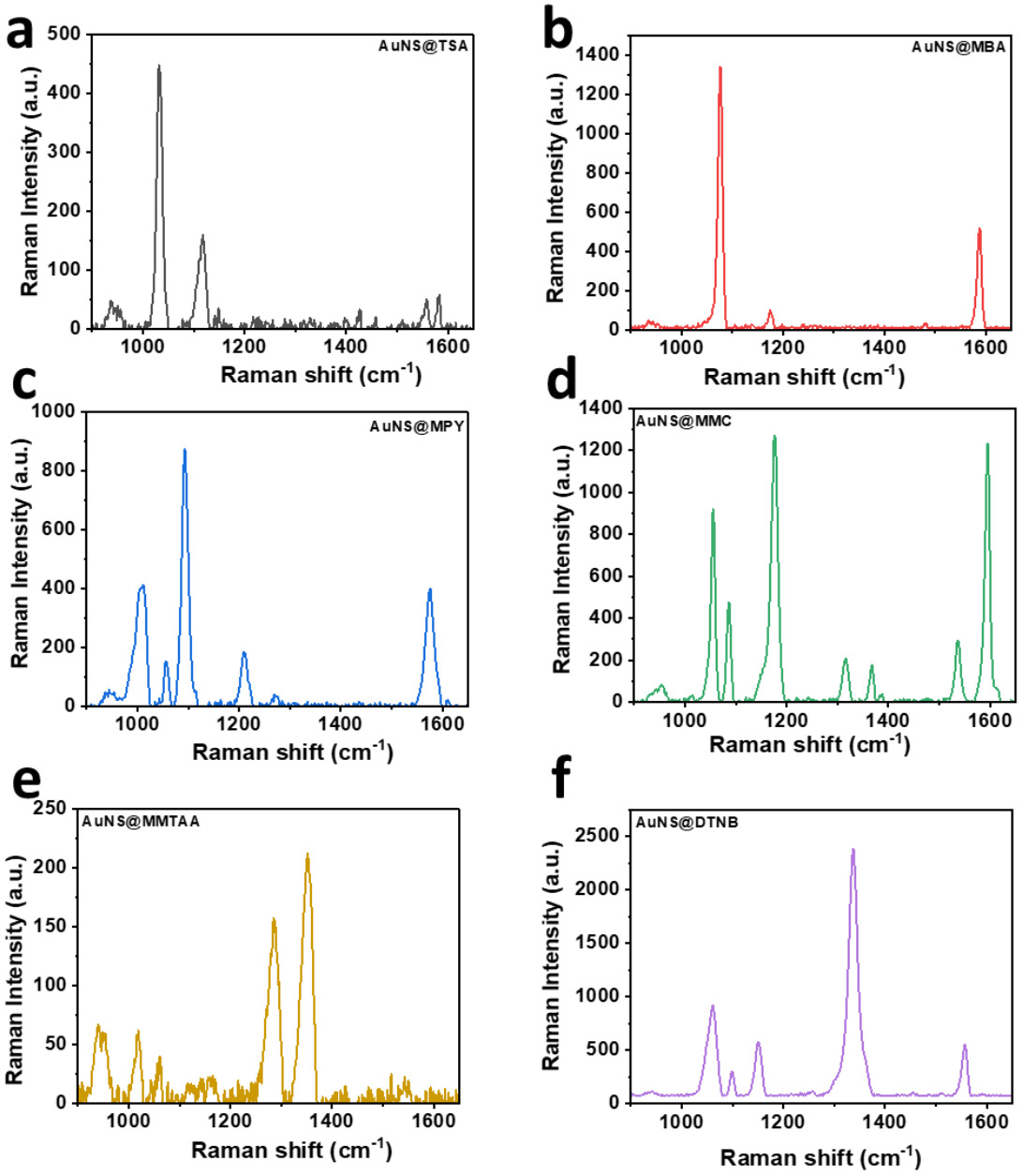


Figure S6. SERS spectra of AuNS coated with seven Raman reporter molecules. The most significant peak of each SERS nanotag was recorded, including (a) 1032 cm^{-1} of AuNS@TSA (b) 1077 cm^{-1} of AuNS@MBA (c) 1093 cm^{-1} AuNS@MPY (d) 1176 cm^{-1} of AuNS@MMC (e) 1351 cm^{-1} of AuNS@MMTAA (f) 1337 cm^{-1} of AuNS@DTNB (g) 1378 cm^{-1} of AuNS@TFMBA

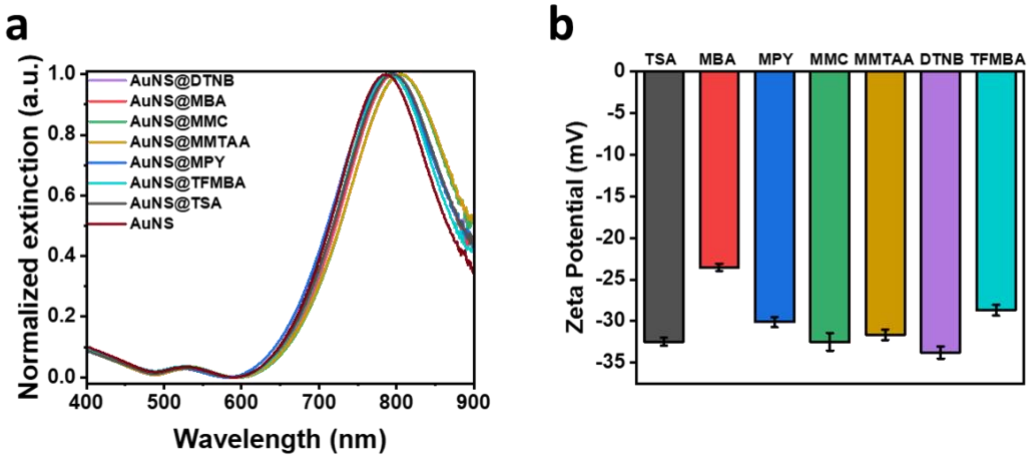


Figure S7. UV-Vis spectra (a) and zeta potential (b) of seven different SERS nanotags. UV-Vis spectra of AuNSs presented red shifts after conjugation with Raman reporter molecules (a) and all SERS nanotags had negative charges (b)

Table S2. 21 SERS spectra of two types of SERS nanotags

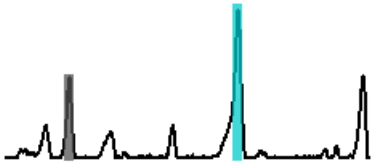
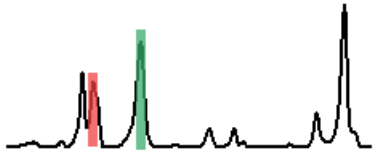
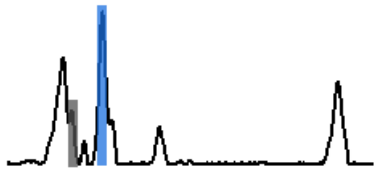
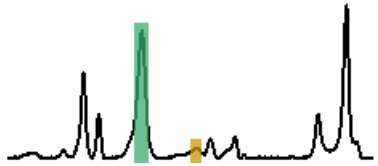
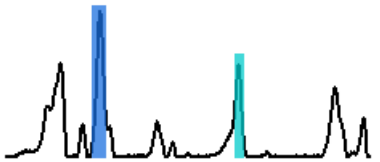
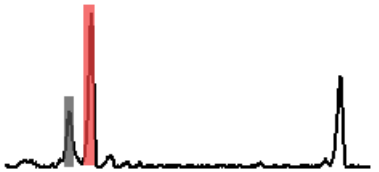
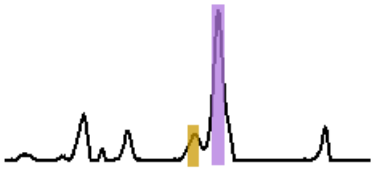
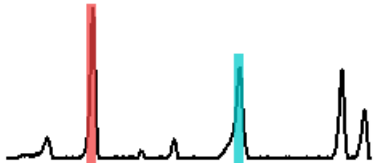
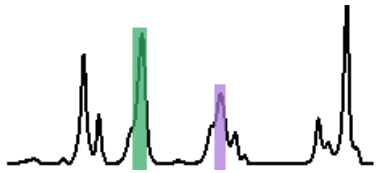
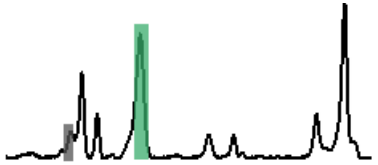
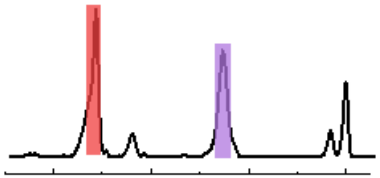
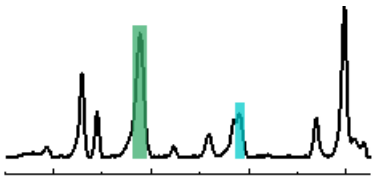
Spectra	Spectral barcode	Spectra	Spectral barcode
	 1000001		 0101000
	 1010000		 0001100
	 0010001		 1100000
	 0000110		 0100001
	 0001010		 1001000
	 0100010		 0001001

Table S2. 21 SERS spectra of two types of SERS nanotags (continued)

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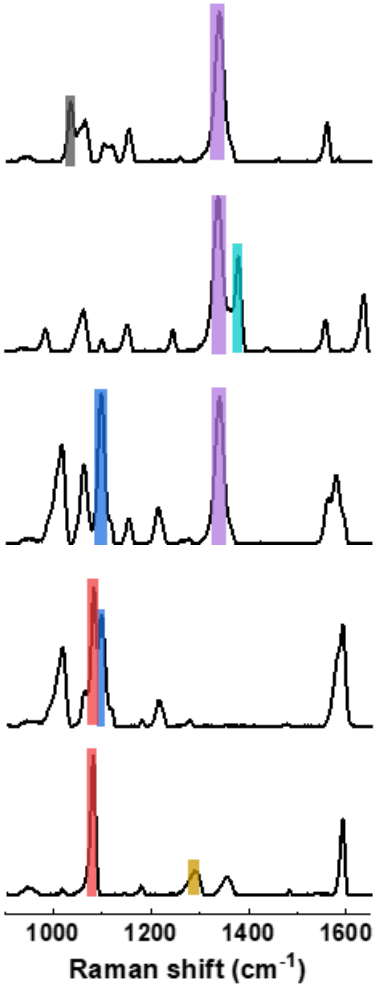
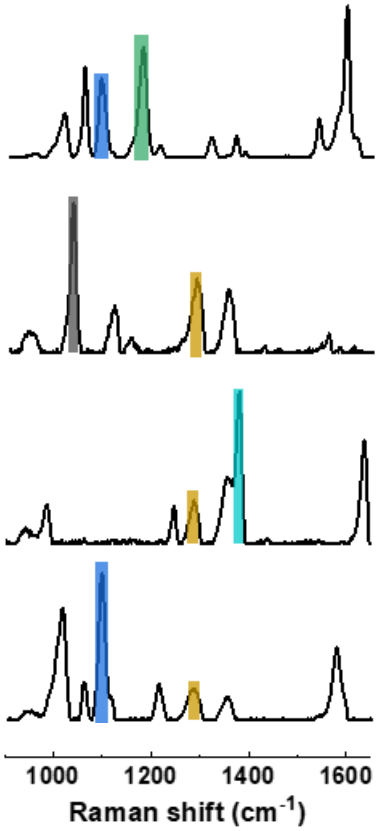
	 1000010		 0011000
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Table S3. 35 SERS spectra of three types of SERS nanotags

Spectra	Spectral barcode		Spectra	Spectral barcode	
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		1001001			0101001
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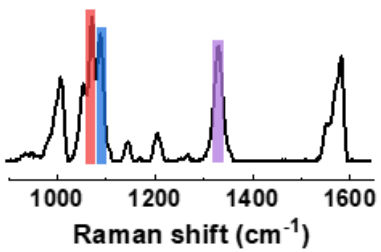
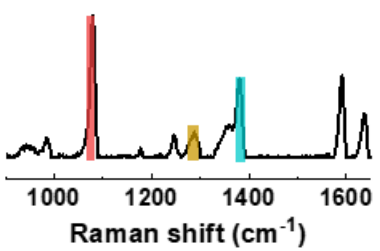
		0110010			0100101

Table S3. 35 SERS spectra of three types of SERS nanotags (continued)

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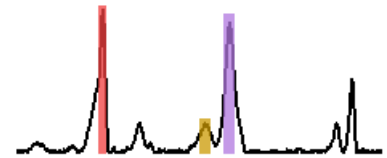
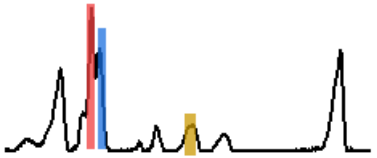
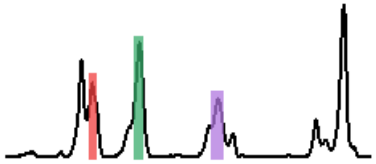
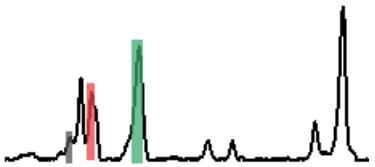
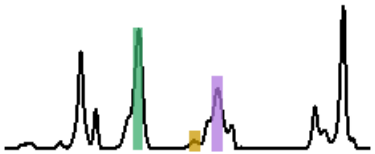
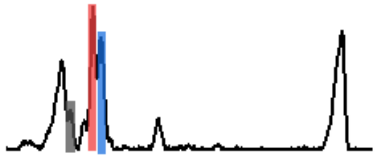
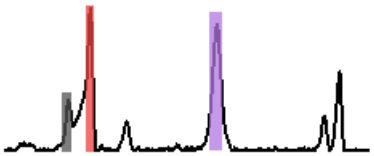
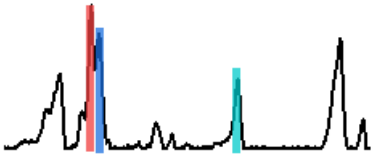
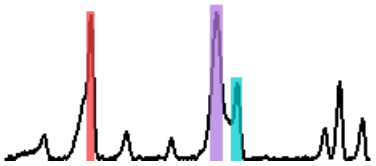
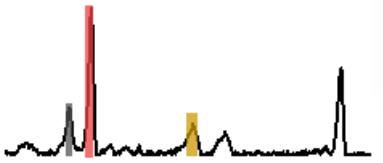
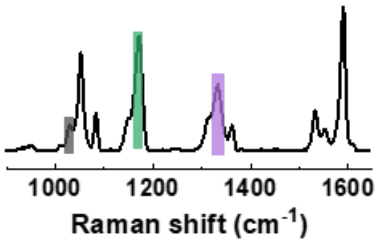
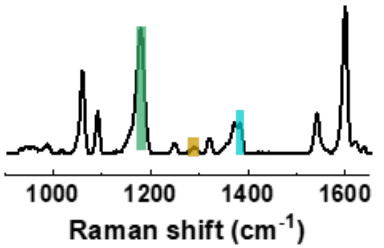
		0100110			0110100
		0101010			1101000
		0001110			1110000
		1100010			0110001
		0100011			1100100
		1001010			0001101

Table S3. 35 SERS spectra of three types of SERS nanotags (continued)

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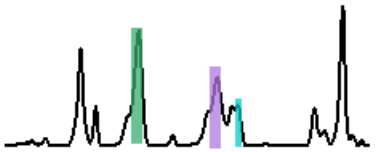
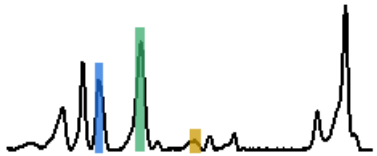
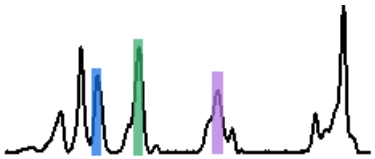
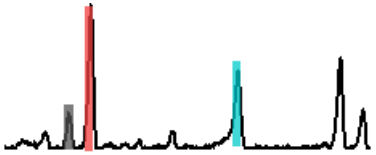
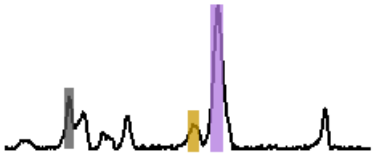
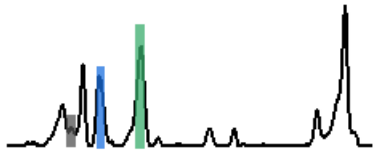
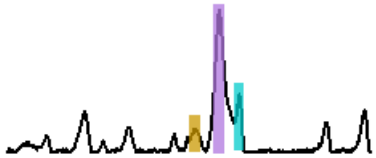
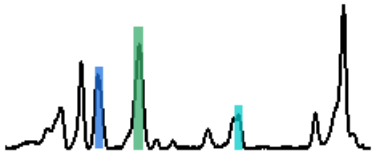
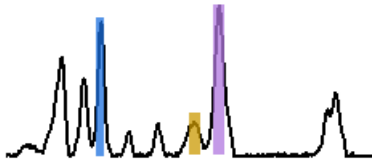
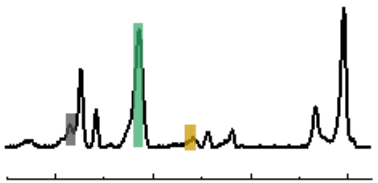
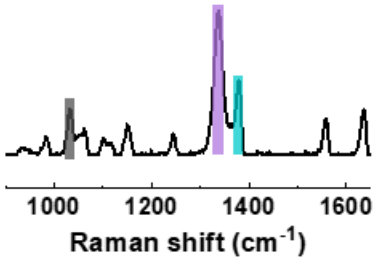
		0001011			0011100
		0011010			1100001
		1000110			1011000
		0000111			0011001
		0010110			1001100
		1000011			

Table S4. 35 SERS spectra of four types of SERS nanotags

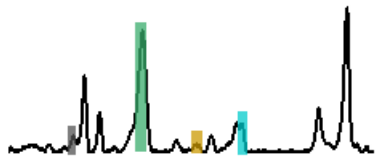
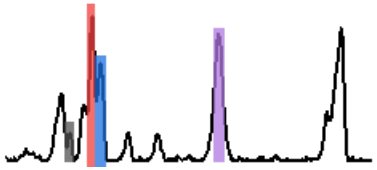
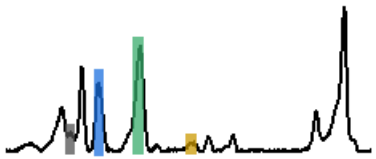
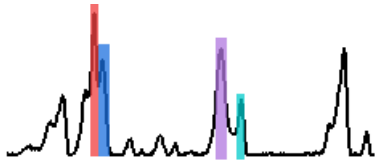
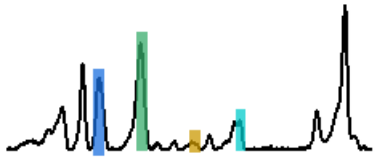
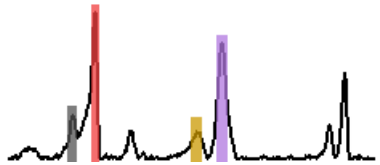
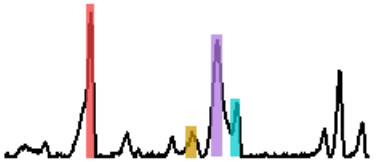
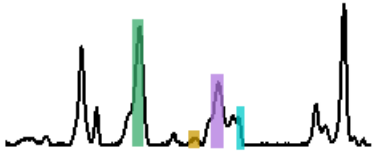
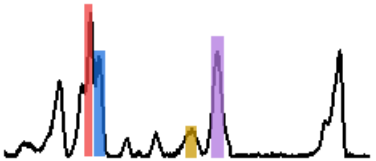
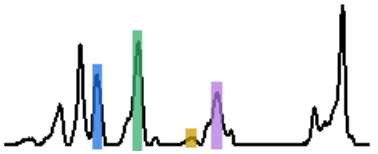
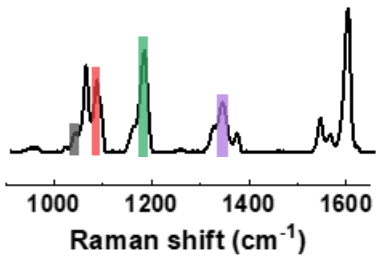
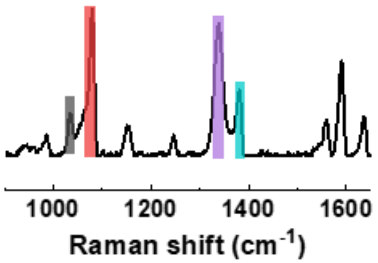
Spectra	Spectral barcode		Spectra	Spectral barcode	
		1001101			1110010
		1011100			0110011
		0011101			1100110
		0100111			0001111
		0110110			0011110
		1101010			1100011

Table S4. 35 SERS spectra of four types of SERS nanotags (continued)

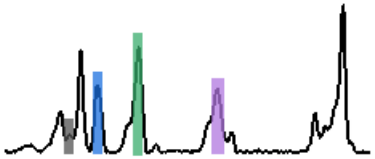
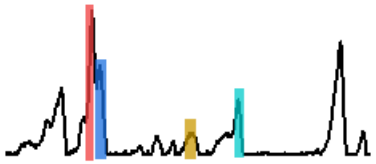
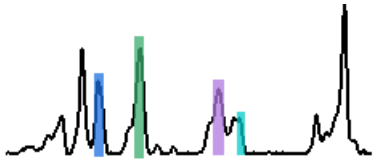
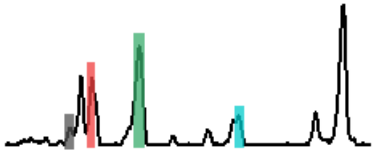
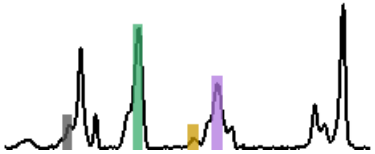
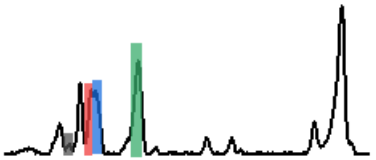
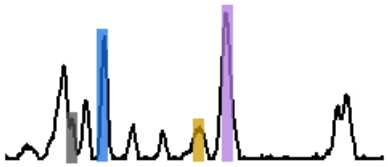
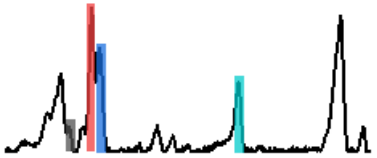
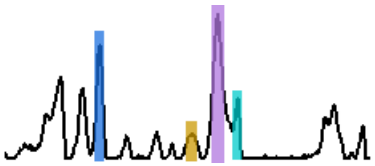
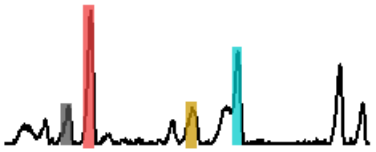
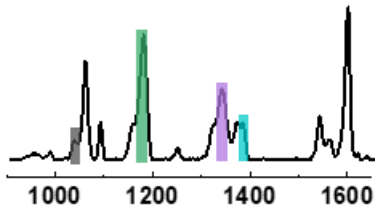
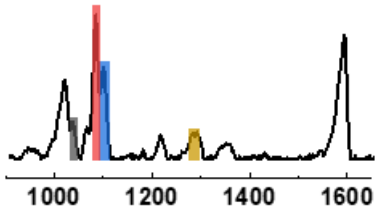
Spectra	Spectral barcode		Spectra	Spectral barcode	
		1011010			0110101
		0011011			1101001
		1001110			1111000
		1010110			1110001
		0010111			1100101
		1001011			1110100

Table S4. 35 SERS spectra of four types of SERS nanotags (continued)

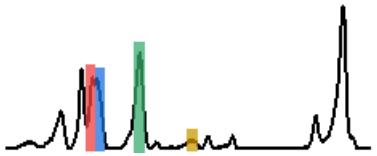
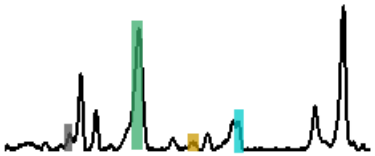
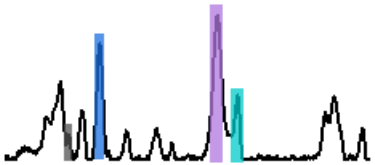
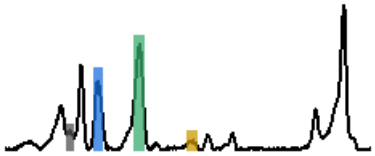
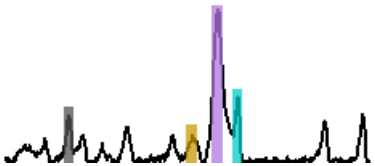
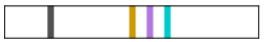
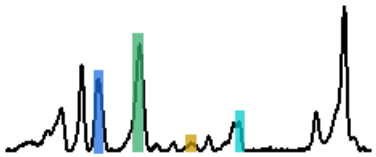
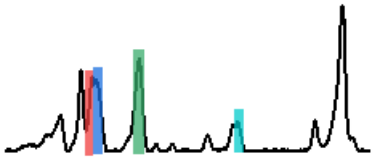
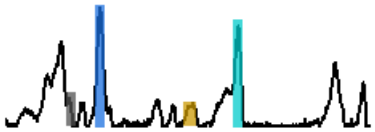
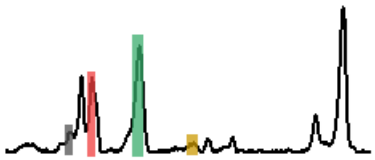
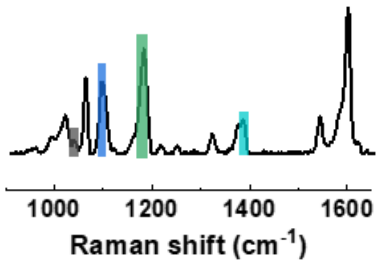
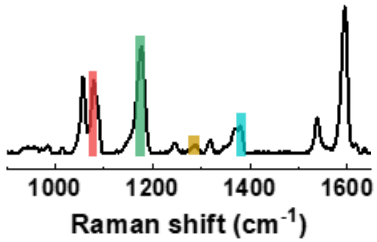
Spectra	Spectral barcode		Spectra	Spectral barcode	
		0111100			1001101
		1010011			1011100
		1000111			0011101
		0111001			1010101
		1101100			1011001
		0101101			

Table S5. 21 SERS spectra of five types of SERS nanotags

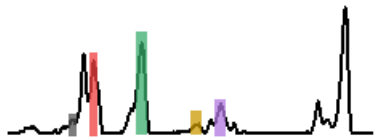
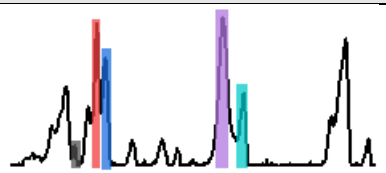
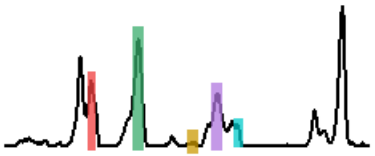
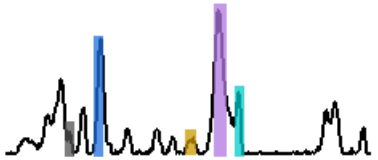
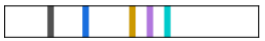
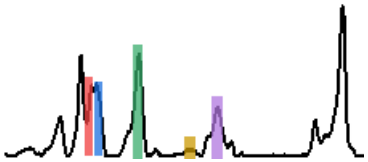
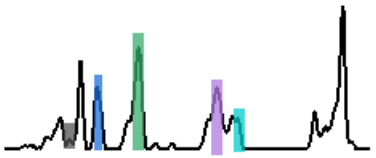
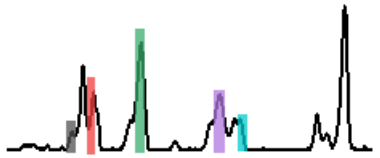
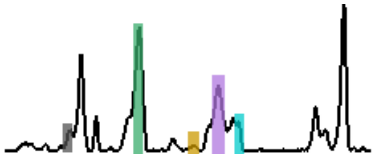
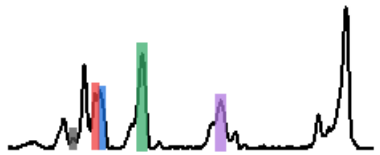
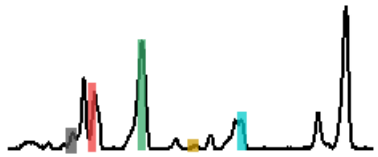
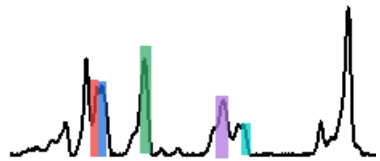
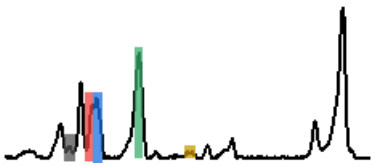
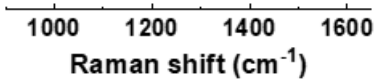
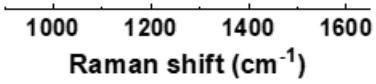
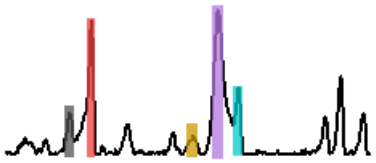
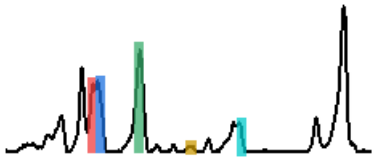
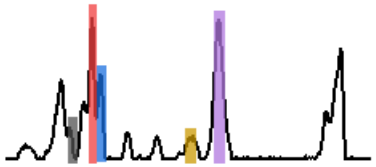
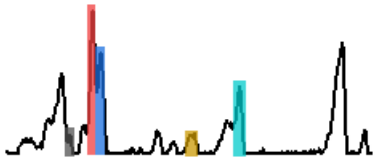
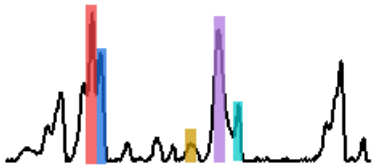
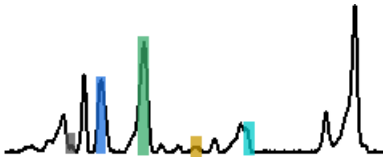
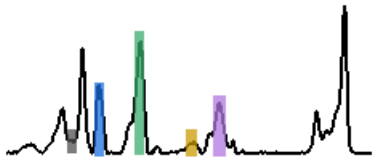
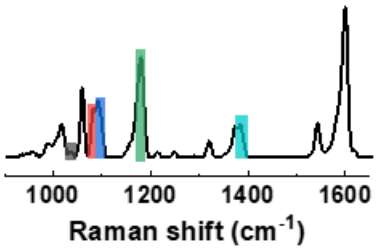
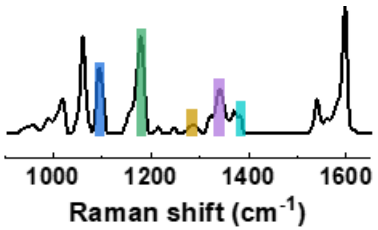
Spectra	Spectral barcode		Spectra	Spectral barcode	
		1101110			1110011
		0101111			1010111
		0111110			1011011
		1101011			1001111
		1111010			1101101
		0111011			1111100
					

Table S5. 21 SERS spectra of five types of SERS nanotags (continued)

Spectra	Spectral barcode		Spectra	Spectral barcode	
		1100111			0111101
		1110110			1110101
		0110111			**1011101**
		1011110			1111001
		0011111			

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