Electronic Supplementary Information

Intracellular optical probing with gold nanostars

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Figure S1. Size distribution based on TEM data of the gold nanostars synthesized with (A) 75 mM, (B) 50 mM and (C) 25 mM HEPES. Average and standard deviation were obtained from (A) 28 particles, (B) 126 particles and (C) 187 particles.



Figure S2: SERS spectra of (A) aggregated nanostars, and (B) nanostars incubated for 24 h in DMEM-FCS. Excitation wavelength: 785 nm, intensity: 3×10^5 W/cm², acquisition time per spectrum: 1s (Color code: red: 25 mM HEPES; blue: 50 mM HEPES; black: 75 mM HEPES). Bands labeled with (H) are assigned to vibrations of unmodified HEPES.¹ Scale bars: (A) 50 cps, (B) 20 cps.



Figure S3. (A) SERS spectra of different concentrations of pMBA with gold nanostars synthesized with 25 mM HEPES (scale bar 50 cps). Excitation wavelength: 785 nm, intensity: 3×10^5 W/cm², acquisition time per spectrum: 1s. The band at 521 cm⁻¹ is assigned to in-plane ring deformation, 1078 cm⁻¹ to the ring breathing mode, 1177 cm⁻¹ to the C-H in plane bending, and 1587 cm⁻¹ to the ring stretching mode.² (B) SERS signal of the ring stretching band for different pH values (scale bar 200 cps). (C) SERS signal for two pH values showing at acid pH the band at 1702 cm⁻¹ assigned to COOH stretching, and at basic pH the band at 1422 cm⁻¹ assigned to COO⁻ stretching (scale bar 100 cps). The band in the range of 1300-1500 cm⁻¹ in the spectra of (A) and (C) is due to the optical configuration during the particular experiment.



Figure S4. UV-vis-NIR spectra of gold nanostars synthesized with (A) 25 mM, (B) 50 mM, (C) 75 mM HEPES, in the absence (full line) and presence of (dashed lines) pMBA at the indicated concentration.



Figure S5: SERS spectra of different concentrations of adenine with gold nanostars synthesized with 25 mM HEPES (A) in the absence and (B) in the presence of NaCl (scale bar 50 cps). Excitation wavelength: 785 nm, intensity: 3×10^5 W/cm², acquisition time per spectrum: 1s. (C) Molecular structure of adenine. Numbering of atoms refers to the band assignment description in Table S1. The band in the range of 1300-1500 cm⁻¹ in the spectra of (A) is due to the optical configuration during the particular experiment.

stretching.	
Raman shift (cm ⁻¹)	Band assignment
624-629	6-ring def in-plane
733-738	Ring br in-plane
964-973	5 ring def in-plane

Table S1. Raman shifts and band assignments for the spectra of adenine shown in Figure S5. Assignments are based on ref 3. Abbreviations: def, deformation; br, breathing; bend, bending; str, stretching.

1275	Bend C8-H, N9-H, str N7-C8		
1344	Bend C2-H, C8-H, N9-H, str C6-N1, C8-N9, N3-C4		
1378	Bend C2-H, N9-H, str C8-N9, C4-N9		



Figure S6. Electric field intensity distribution (normalized to the intensity of the incident field) at 785 nm in the xy plane for two spherical nanoparticles of 22 nm of radius separated by a 2 nm gap as obtained in an FDTD simulation. The excitation wavelength was set to 785 nm. The polarization of the incident plane wave was in the x direction. The monitor was placed in the equatorial plane. The color scale bar is in the same range as the scale for Figure 4C and 4D in the manuscript text, to enable comparison.

Raman shift (cm ⁻¹)	Tentative band assignment	Raman shift (cm ⁻¹)	Tentative band assignment	
424	Trp	1489	Amide II, NH ₃ ⁺	
460	Protein S-S str, C-S str, Trp ring def	1540	Amide II, lipid CH2 sciss	
503	Protein S-S str	1556	Amide II; Trp, Tyr, COO ⁻ str	
625	C-S str, Tyr C-C twist, Phe	1580	Amide II, C-C str,COO- str	
659	Cys C-S str	1590	C=C str, COO ⁻ str	
677	C-S str, His imidazole ring def			
809	Pro, Tyr, C-C str, O–P–O str			
840	Tyr ring br, Phe, Cα-N and C-C str, O-P-O str			
975	Lipids C-C str, Pro, Val	Terr terreterilisen Terret	Discontraction Com	
1003	Phe ring br	cysteine; Pro, proline; Val, valine; His, histidine; A, adenine; C, cytosine; G, guanine; str, stretching; def, deformation; twist, twisting; br, breathing; bend, bending;		
1059	C-C and C-N str			
1123	Lipid C-C str wag, wagging; sciss, scissor		soring	
1134	Protein backbone C-C str; C-N str			
1171	Tyr and lipids C-H bend			
1185	Lipids, phosphate			
1199	Aromatic C-O and C-N			
1228	Amide III; Trp ring			
1235	Amide III, CH ₂ wag, O-P-O str			
1274	Amide III; CH/CH ₂ /CH ₃ def			
1300	Amide III, A and C, CH/CH ₂ /CH ₃ def			
1315	Amide III, G, lipids CH ₂ /CH ₃ def			
1325	Amide III; G; protein CH ₂ /CH ₃ twist			
1340	Lipid and protein CH ₂ /CH ₃ wag			
1345	CH def			
1353	Protein CH/CH ₂ /CH ₃ def; Trp			
1365	Lipids CH ₂ def			
1415	COO ⁻ str			
1421	A, G, CH ₃ CH ₂ twist			
1432	CH ₂ def			
1439	Lipid and protein CH2 def			

Table S2. Raman shifts and their tentative assignments in the spectra displayed in Figures 5 and 6. Band assignments based on refs 4-10.



Figure S7. Slices of X-ray tomographic reconstruction (further examples) of (A) 3T3 and (B) HCT-116 cells incubated for 24 h with gold nanostars synthesized with 25mM HEPES (cf. Figure 1B). The red arrows indicate single particles or aggregates of nanostars. Abbreviations: Nu nucleus; NM nuclear membrane; PM plasma membrane; V vesicles; M mitochondria; L lipid droplets. Scale bars: $2 \mu m$.

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