Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Label-free Chemical Imaging of Live Fibroblasts by SERS Effective Nanofilms

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Theoretical calculation of LFI EFs and SERS EFs of Ag-PAA nanoparticles (oblate or prolate) on silica, in water in comparison with gold (instead of silver) and SERS spectra of live NIH/3T3 fibroblasts with embedded SiO_2@Ag-PAA particles.

UV-Vis absorbance spectra and TEM images of Ag-PAA NPs before and after heating at 37ºC, TEM images of Ag NPs prepared by the Meisel's procedure before and after heating at 37ºC, fluorescence spectra of SiO_2@Ag and SiO_2@Ag-PAA particles before and after heating at 37ºC, optical phase contrast images of live NIH/3T3 fibroblasts before and after electroporation with the plotted statistical data, confocal laser scanning microscopy (CLSM) image (transmission mode) of a selected single live NIH/3T3 fibroblasts with embedded SiO_2@Ag-PAA particles, CLSM and Raman spectroscopic images of a selected single live NIH/3T3 fibroblast used for chemical imaging, SERS spectra of SiO_2@Ag-PAA particles in CM or PBS without fibroblasts and CLSM images of SiO_2@Ag-PAA particles in PBS.

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Table 1. Theoretical calculation of LFI EFs and SERS EFs of Ag-PAA nanoparticles (oblate or prolate) on silica, in water in comparison with gold (instead of silver).

	(3,1) Ag (400)/silica	**(3,1) Au (530)/silica**	**(3,1) Ag (400)/water**	**(3,1) Au (530)/water**	**(1,3) Ag (400)/silica**	**(1,3) Au (530)/silica**	**(1,3) Ag (400)/water**	**(1,3) Au (530)/water**
β_1	-0.88+0.02i	-0.91 + 0.39i	-1.70 + 0.08i	-1.59 + 1.23i	2.19 + 0.16i	1.10 + 0.67i	1.00 + 0.03i	0.78 + 0.20i
β_3	-7.91+2.39i	0.54 + 2.38i	2.34 + 0.16i	1.30 + 0.63i	-1.10 + 0.04i	-1.07 + 0.61i	-2.71 + 0.22i	-1.40 + 2.49i
A_1^{perp}	1.88	3.21	12.64	21.53	11.57	5.43	4.41	3.52
A_1^{para}	1.66	1.70	2.41	2.47	10.20	2.87	0.84	0.40
A_3^{perp}	164.01	19.33	24.07	11.14	2.91	4.99	32.41	43.49
A_3^{para}	144.54	10.22	4.60	1.27	2.57	2.64	6.19	4.99
$M_1^{loc/perp}$	0.13	0.23	0.93	1.59	8.54	4.01	3.25	2.60
$M_1^{loc/para}$	1.53	1.57	2.23	2.28	2.67	0.75	0.22	0.10
M_1^{loc}	1.67	1.81	3.17	3.88	11.21	4.76	3.47	2.70
$M_3^{loc/perp}$	75.92	8.95	11.14	5.15	0.38	0.65	4.24	5.69
$M_3^{loc/para}$	77.63	5.49	2.47	0.68	2.23	2.29	5.38	4.33
M_3^{loc}	153.55	14.44	13.61	5.84	2.61	2.95	9.63	10.03
FE_{41}^{perp}	0.08	0.23	3.68	10.67	84.11	18.56	12.20	7.79
$F_0E_4^1$	2.81	3.33	11.92	21.45	125.95	23.27	13.13	8.13
FE_{43}^{perp}	8.82e+03	122.67	190.05	40.73	0.48	1.42	59.77	107.65
FE_4^3	2.36e+04	218.10	228.46	45.23	6.84	8.92	120.07	159.63

The indexes *1* and *3* indicate the incident polarization along *x* and *z* axes. The abbreviators "*loc*" is local, "*para*" is parallel and "*perp*" is perpendicular.

Table 2. SERS spectra (300 cm^{-1} - 1000 cm^{-1}) of live NIH/3T3 fibroblasts with embedded SiO_2@Ag-PAA particles. The cited references can be found from the general database entitled "Raman spectroscopy of biological tissues".[39]

Nucleus		Nucleus membrane				Cytoplasm			Assignment
2	3	1	4	5	9	6	7	8	
						303m	-	312w	Phospholipids[56]
						350s	-	355w	Phospholipids[56]
						415w	-	400w	Phosphatidylinositol[42]
						470m	-	-	Polysaccharides, amylose, amylopectin[39]
						534s	-	-	Cholesterol ester[42]
						548s	-	-	Cholesterol[42]
						561s	-	-	Phospholipids[56]
						584s	-	-	Phospholipids[56]
610w	w	vw	vw	611	vw	vw	w	611vs	Cholesterol[42]
		-	-	655	-	643w	-	w	C-C twisting of tyrosine[23,31]
						677m	-	-	Ring breathing in the DNA bases[72]
						719w	-	-	Symmetric stretching of choline group, phospholipids[42]
768w	w	vw	vw	773	vw	772vs	m	s	Phosphatidylinositol[42]
						892m	-	-	Saccharide band, C-C skeletal backbone[42,72]

vs (very strong), **s** (strong), **m** (moderate), **w** (weak), **vw** (very weak)

Table 3. SERS spectra (1000 cm^{-1} - 2000 cm^{-1}) of live NIH/3T3 fibroblasts with embedded SiO_2@Ag-PAA particles. The cited references can be found from the general database entitled "Raman spectroscopy of biological tissues".[39]

Nucleus		Nucleus membrane				Cytoplasm			Assignment
2	3	1	4	5	9	6	7	8	
						918w	-	-	RNA mode[72]
		-	-	930w	-				ν(C-C) amino acids[45]
						943w	-	-	polysaccharides, amylose[39]
						975w	-	-	RNA mode[72]
						1000w	-	-	Phenylalanine[43]
		-	-	1010w	-	1008w	-	-	polysaccharides, pectin[59,39]
						1026w	w	1030w	Carbohydrates, phenylalanine of collagen[3,31]
						1047w	w	w	Glycogen[65]
						1069w	1060w	w	PO_2^- stretching (DNA/RNA)[73]
		-	-	1084w	-				Phosphodiester groups in nucleic acids[3]
						1086m	-	m	ν(C-C) *gauche*[41]
1129w	1125w					1125m	-	w	ν(C-C) in lipid[31]
		1130m	-	m	-				C-C skeletal stretch [66]
1180w	1184w	w	w	1184s	w	1180s	1184w	1184m	Cytosine, guanine, adenine[62,33]
						-	1230m	-	Antisym phosphate[3]
						1264w	1264w	1268w	Triglycerides. phospholipids[54,41]
1306m	1310m	w	m	1310s	w	1310vs	vw	m	CH_3/CH_2 of lipid/collagen[31]

1360s		m	m	1364vs	m	1360vs	m	vs	Tryptophan[39,31]
1417w									C=C in quinoid ring[75]
1446w		-	-	1420w	-	1430w	w	w	CH deformation (DNA/RNA/carbohydrates),CH_2 (proteins/lipids)[73,23,24]
						1466w	w	w	Lipids[3]
1507vs		m	m	1512vs	m	1512vs	w	vs	Cytosine[62]
1564s	1572s								Tryptophan[45]
		m	m	1576vs	m	1573s	-	s	Guanine (N_3), adenine, TRP (protein)[62,23]
						-	-	1594vw	C=N and C=C in quinoid ring[59]
1650vs		1648m	1648m	1652vs	1648m	1648vs	vw	vs	Amide I lipid C=C[92,23,43]

vs (very strong), **s** (strong), **m** (moderate), **w** (weak), **vw** (very weak)

Table 4. SERS spectra (2000 cm^{-1} - 3800 cm^{-1}) of live NIH/3T3 fibroblasts with embedded SiO_2@Ag-PAA particles. The cited references can be found from the general database entitled "Raman spectroscopy of biological tissues".[39]

Nucleus		Nucleus membrane				Cytoplasm			Assignment
2	3	1	4	5	9	6	7	8	
						-	-	2427	OH-NH-CH stretching[78]
		-	-	2545	-				ν(S-H) aminoacid methionine[39]
		-	-	2720	-	-	-	2721	Stretching of CH, NH, OH groups[42]
2780	2780					-	-	2782	Stretching of CH, NH, OH groups[42]
2815	2815	-	-	2828	-	-	-	2828	CH, CH_2, CH_3 (change in the amount of lipids)[68,90]
2873	2873	vw	vw	2873	vw	-	-	2868	CH, CH_2 of lipids and proteins[68]
2925	2925					-	-	2929	Symmetric CH_3 of proteins[29]
		-	-	2960	-				Out-of-plane chain end antisymmetric CH_3 stretch[42]
3004	3004	vw	vw	3014	vw	-	-	3012	C-H of lipids and fatty acids[39,71]
-	3077					-	-	3062	C-H of lipids and fatty acids[39,71]
-	3162	vw	vw	3162	vw	-	-	3156	C-H of lipids and fatty acids[39,71]
-	3212								OH, NH stretching[53,42]
-	3297	vw	vw	3220	vw	-	-	3223	OH, NH stretching[53,42]
		-	-	3304	-				OH stretching[94]

vs (very strong), **s** (strong), **m** (moderate), **w** (weak), **vw** (very weak)

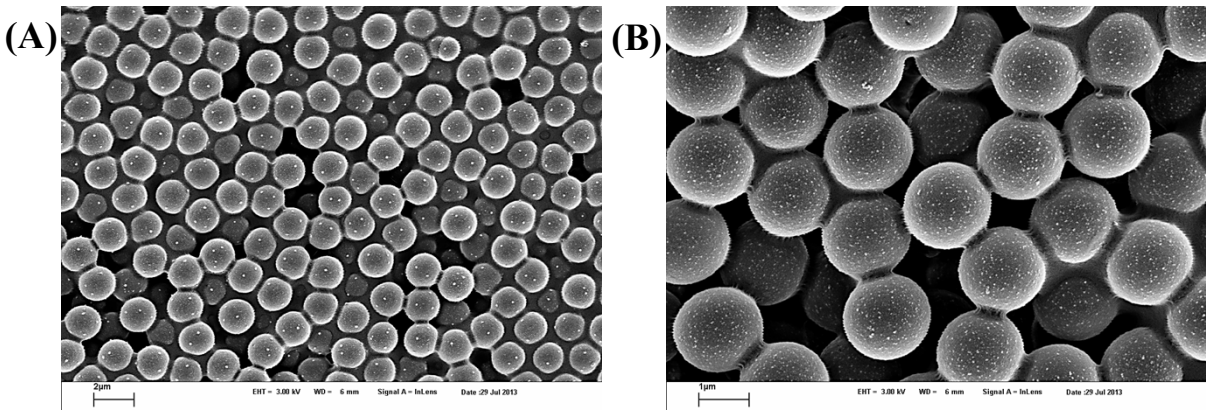

Figure S.1 SEM images of colloidal silica after adsorption of (A) silver-citrate nanoparticles (scale bar is 2 µm) or (B) silver-PAA nanoparticles (scale bar is 1 µm).

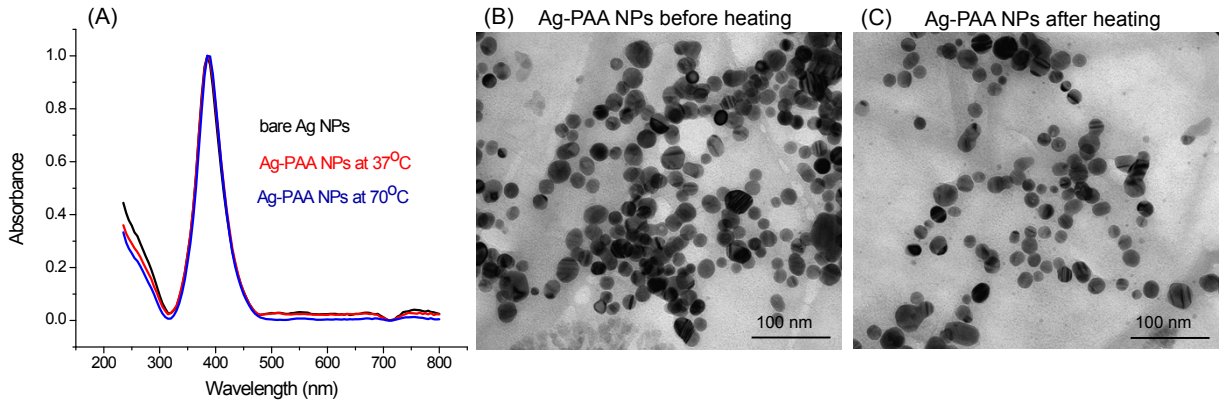

Figure S.2 A) UV-Vis absorbance spectra of silver-citrate nanoparticles in the matrix of polyacrylic acid (PAA) before and after heating at 37ºC in water. B) and C) TEM images of Ag-PAA NPs before and after heating at 37ºC (scale bar is 100 nm).

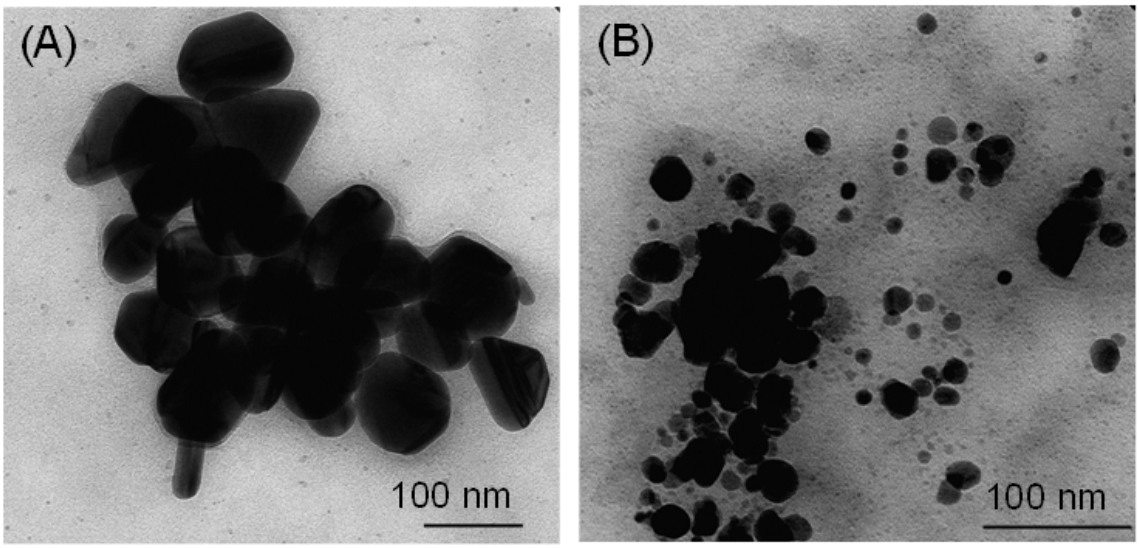

Figure S.3 TEM images of silver-citrate nanoparticles formed by a Meisel's procedure (A) before and (B) after heating at 37ºC (scale bars are 100 nm each).

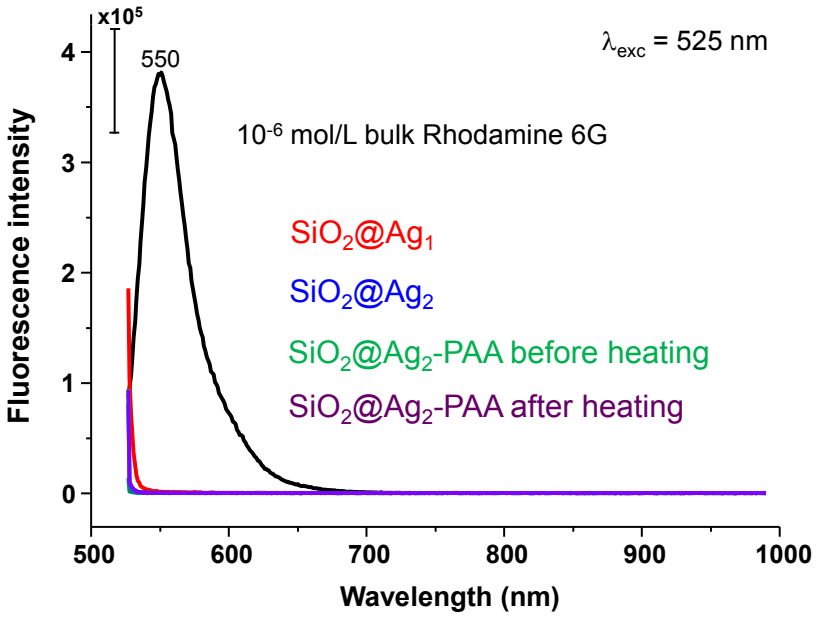

Figure S.4 Fluorescence spectra of Rh6G aqueous solution (10^{-6} mol·L^{-1}) incubated with different Ag NPs adsorbed on silica MPs: SiO_2@Ag_1 (red, Meisel's procedure); SiO_2@Ag_2 (blue, modified Xia's procedure); SiO_2@ Ag_2-PAA (pale blue and violet, Ag NPs in the PAA matrix before and after heating at 37°C). The black curve shows fluorescence spectra of bulk Rh6G aqueous solution (10^{-6} mol·L^{-1}) without silver nanoparticles. The excitation wavelength was 525 nm.

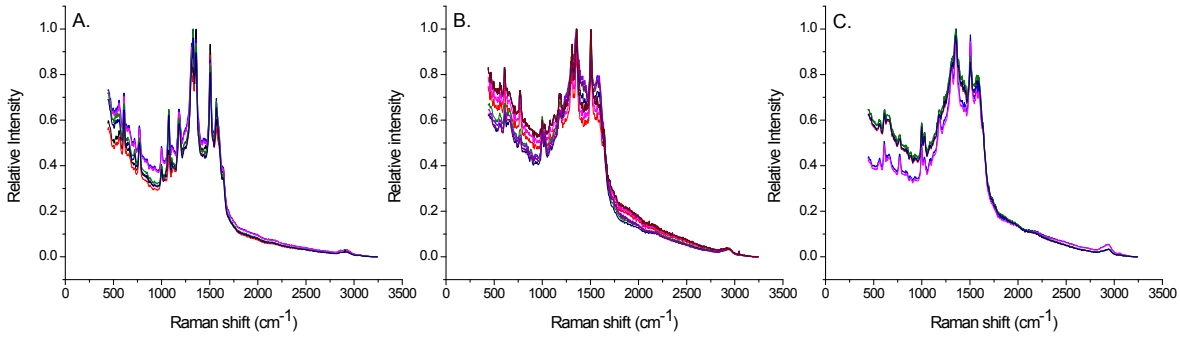

Figure S.5 SERS spectra of Rh6G (10^{-8} mol·L^{-1}) in aqueous solution of SiO_2@Ag-PAA particles as single (A), dimers (B) or closely-packed self-assembly (C). The excitation wavelength was 532 nm with the grating 600 g·mm^{-1} (BLZ = 500 nm) and a spectral resolution of 3 cm^{-1}. The SERS spectra are cumulative and averaged over at least ten particles of each type.

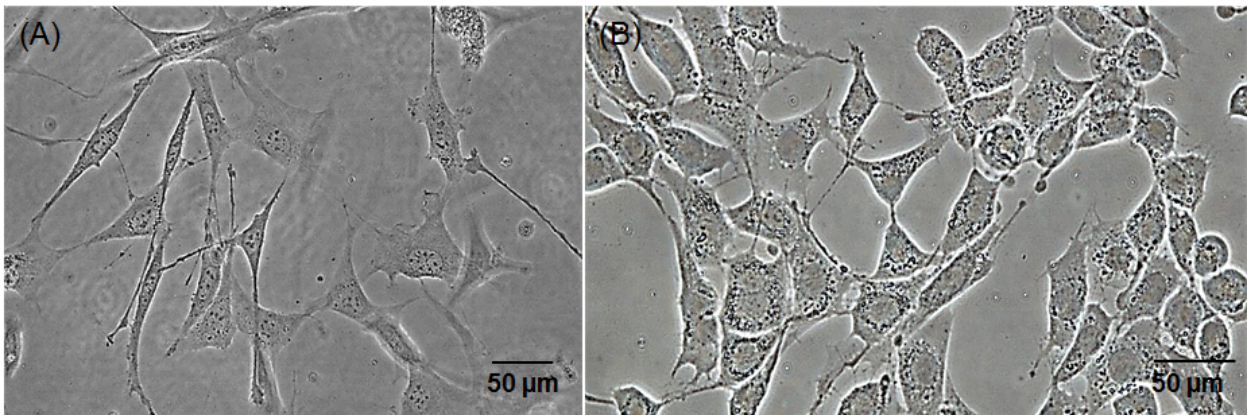

Figure S.6 Optical phase contrast images of live NIH/3T3 fibroblasts (A) before and (B) after electroporation with SiO_2@Ag-PAA particles (scale bars are 50µm).

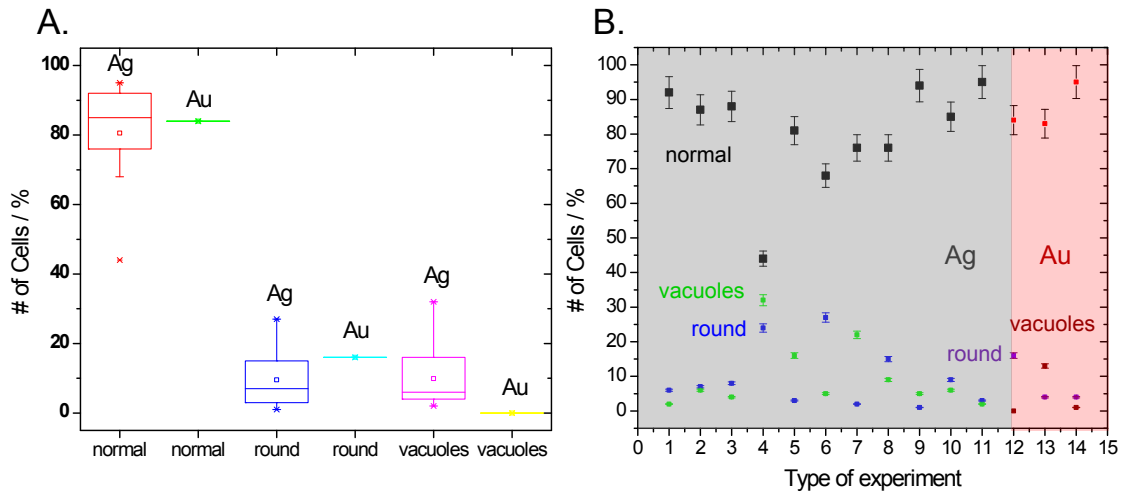

Figure S.7 A) Overall number of live NIH/3T3 fibroblasts (# of Cells, %) with embedded SiO_2@Ag-PAA particles (also those with Au instead of Ag) with different shapes after electroporation. The cell shapes can be differentiated as normal (as before treatment) and those, which acquired round or increased volume of vacuoles after electroporation. (B) Overall number of these cells ((# of Cells, %) at different conditions of electroporation, which are listed below.

Conditions of electroporation	Number of particles			
	several (< 5)	from 5 to 10	from 10 to 20	excess (<40)
± 200 V, 750 ms	2	1	**9**	6
± 250 V, 500 ms	10,13	5,12	**11, 14**	4
± 500 V, 350 ms	3	7	8	-

Below ± 200 V at 750 ms the effect is comparable to incubation/aging.

Above ± 500 V, 350 ms most of the cells were damaged (independent on the number of particles).

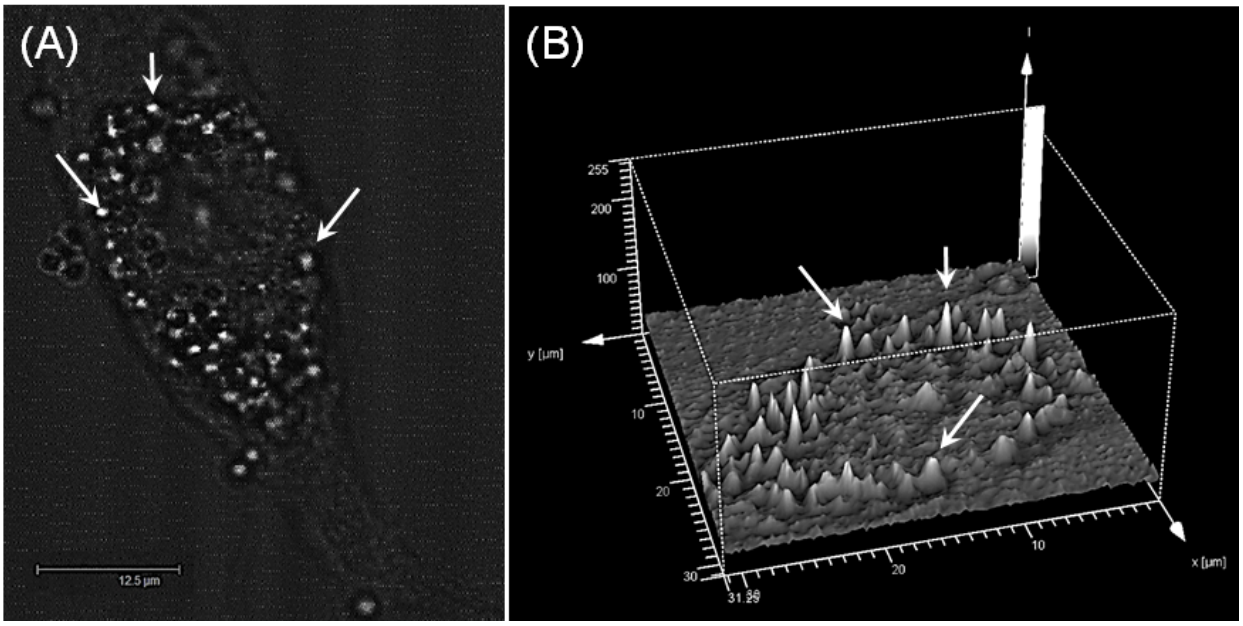

Figure S.8 Confocal laser scanning microscopy (CLSM) image (transmission mode) of a selected single live NIH/3T3 fibroblast with embedded SiO_2@Ag-PAA particles in (A) a 2D mode and (B) 3D mode (scale bar is 12.5 µm). Bright spots are locations of intense scattering as indicated by white arrows.

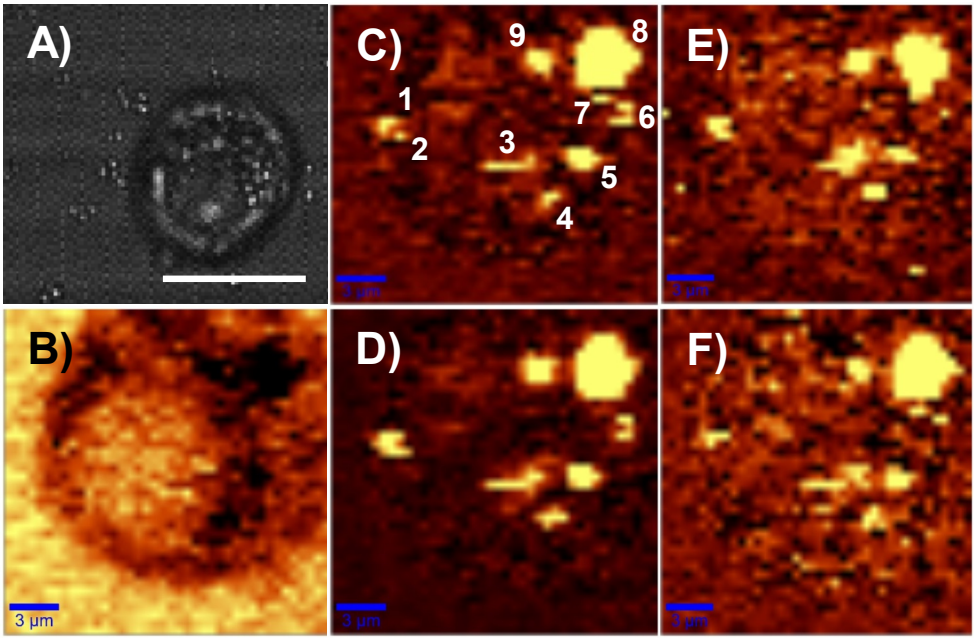

Figure S.9 A) CLSM image (transmission mode) of a selected single live NIH/3T3 fibroblast with SiO_2@Ag-PAA particles (HCA) (scale bar is 8 µm) used for Raman spectroscopic imaging by applying a hypercluster analysis used to construct a coloured image in Figure 7A. For a clear identification of SERS effective colloidal probes, this cell is shown just after the electroporation (prior washing). For all Raman measurements all electroporated cells were triply washed with PBS solution. B)-F) Raman spectroscopic images of this single live NIH/3T3 fibroblast (scale bar is 3 µm), which is constructed by integration of the overall intensity of Raman peaks (B); of the intensity of the strongest bands of phospholipids and cholesterol from 300 to 600 cm^{-1} (C); phosphatidylinositol and cytosine/guanine/adenine from 700 to 1200 cm^{-1} (E); collagen, tryptophan and amide I from 1200 to 2000 cm^{-1} (D) and CH, OH, NH from 2400 to 3300 cm^{-1} (F).

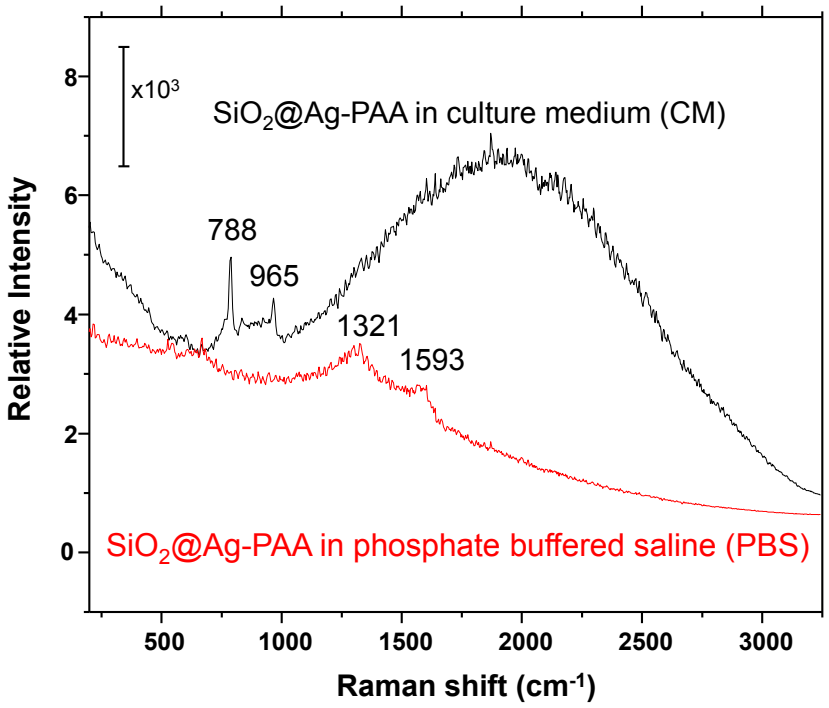

Figure S.10 SERS spectra of culture medium (CM, black) or phosphate buffered saline (PBS, red) bulk aqueous solutions after incubation with SiO_2@Ag-PAA particles without fibroblasts. The laser excitation wavelength was 532 nm and the grating was 600 g·mm^{-1} (BLZ = 500 nm). The broad peak (black curve) is due to the autofluorescence.

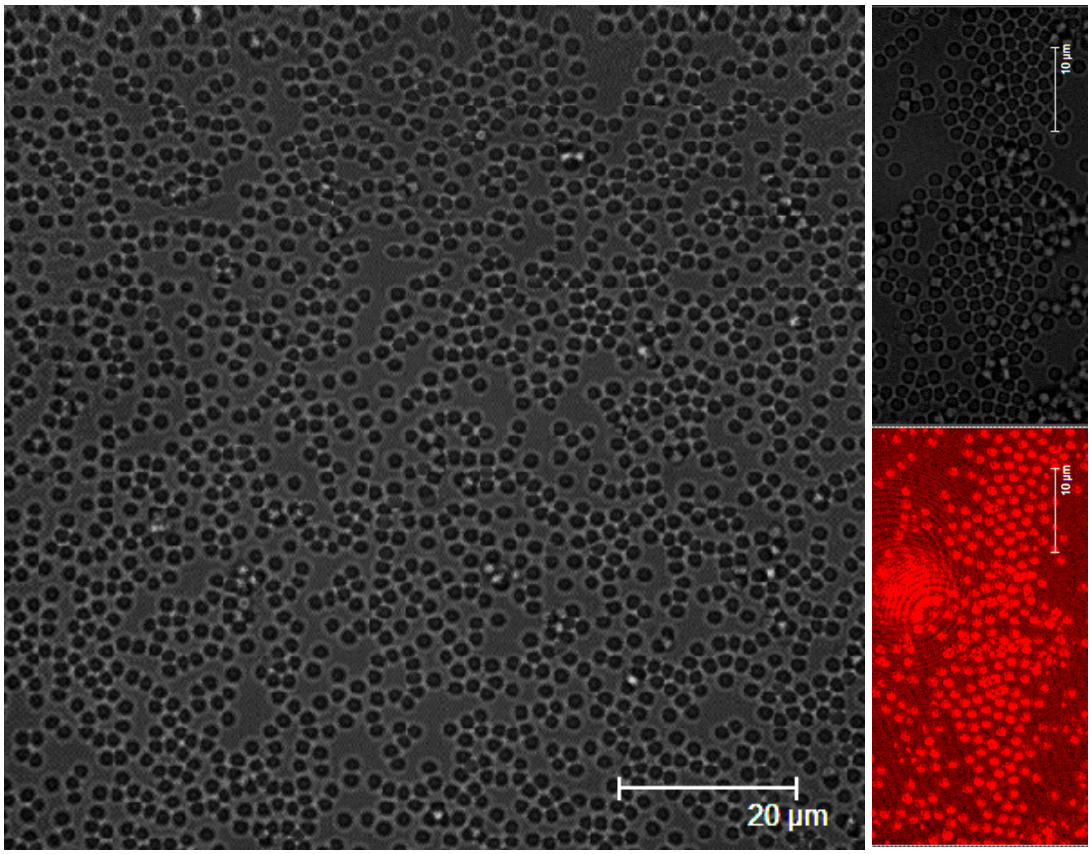

Figure S.11 CLSM images of SiO2@Ag-PAA particles in PBS solution. On the left, CLSM image in transmission mode (scale bar is 20 µm) and on the right, in an excitation mode (λ_{exc} = 514 nm) after labelling with FITC-PAH (scale bar is 10 µm).