Supporting materials of "Planar SERS Nanostructures with Stochastic Silver Ring Morphology for Biosensor Chips**"** (by A.A.Semenova, Eugene A. Goodilin (corresponding author; Russia, Moscow, 119992, Lenin Hills, Chemistry Department, Moscow State University, +7(939)4729 (phone), +7(939)0998 (fax), goodilin@gmail.com), N.A.Brazhe, V.K.Ivanov, A.Ye.Barantchikov, A.E.Goldt, O.V.Sosnovtseva, S.V.Savilov, A.V.Egorov, A.R.Brazhe, E.Y.Parshina, O.G.Luneva, G.V.Maksimov, Yu.D.Tretyakov).

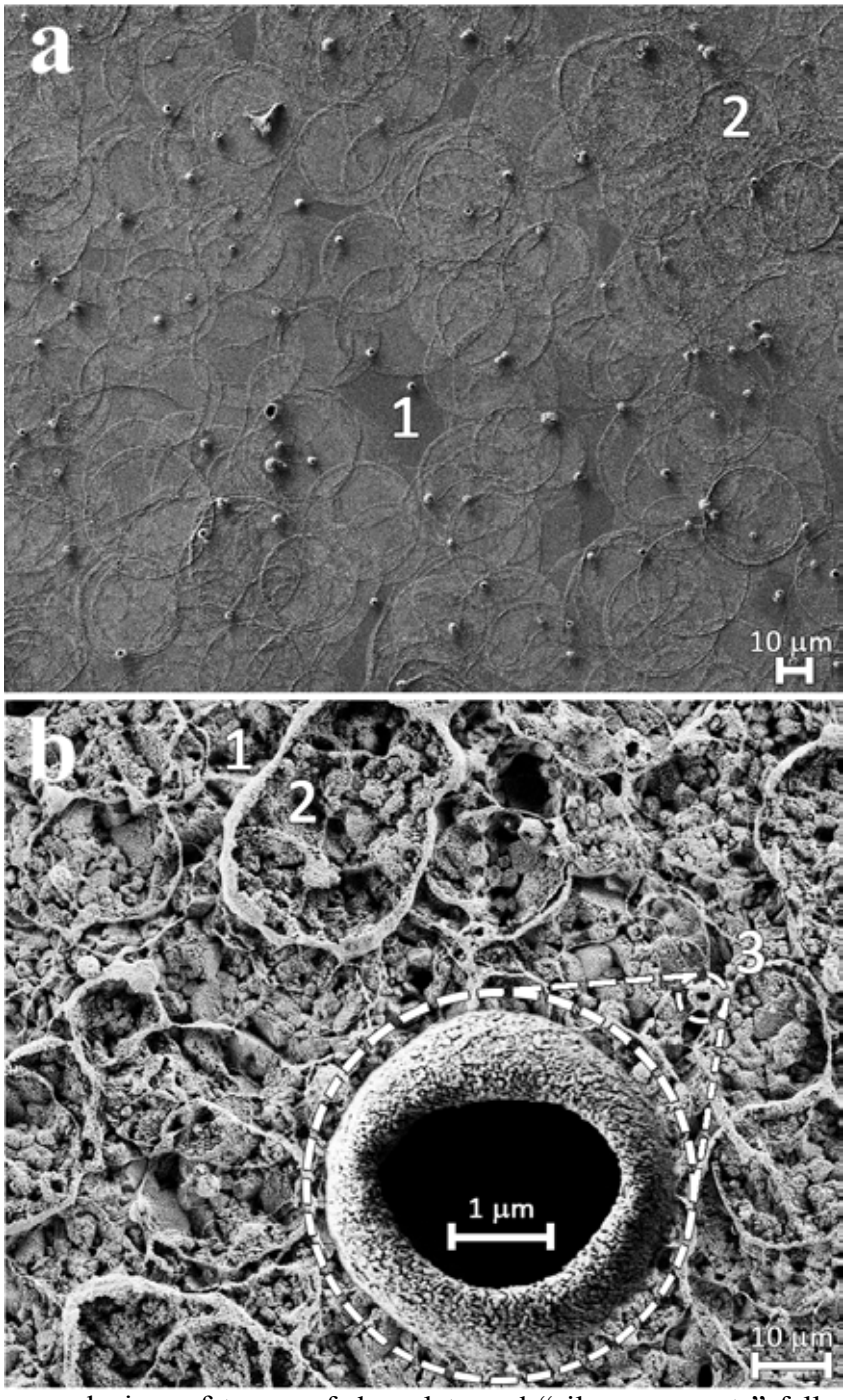

Fig.S1. SEM general view of traces of dropplets and "silver coconuts" fallen onto a glass substrate, 1 - a flat area coated with separate clusters of AgNPs, 2 - intersecting circles made of joint AgNPs surrounding centers of ultrasonic droplet strikes of the substrate ("crater" walls, "coffee rings"), (b) a thicker silver film, 1 - silver shells of evaporated droplets, 2 - nanostructured interior of the droplets, 3 – the inset showing a magnified view of a silver coconut,

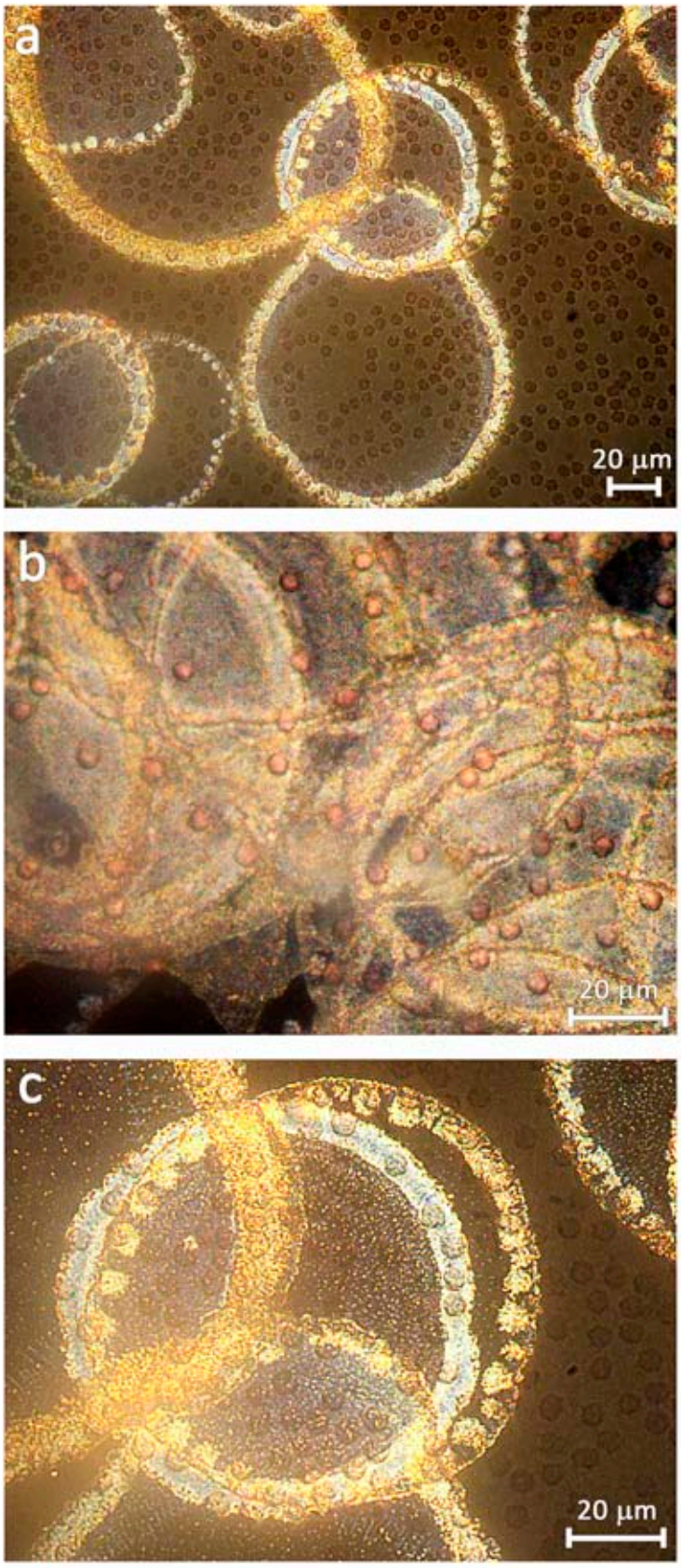

Fig.S2. Optical image of alive erythrocytes immersed in biological buffer and then deposited onto UsSR substrates (reflective mode). "Coffee ring" microstructures consist of nanostructured silver.

Preparation of erythrocyte ghosts and isolated hemoglobine

The erythrocytes samples were prepared from arterial blood taken from male Wistar rats. To compare SERS and RS spectra of erythrocytes and Hb_{cyt}, Hb was isolated from the cytoplasm of erythrocytes from the same blood sample used for the SERS and RS erythrocyte studies. The isolation of Hb was done in a phosphate buffer (4 Na_2HPO_4, 1 NaH_2PO_4 x $2H_2O$) with low osmolarity, pH 7.2. Hemolysis evoked disruption of the erythrocytic plasma membrane and leakage of cytosolic Hb_{cyt} into the buffer. Centrifugation (4500 g, 10 min) was used to separate the supernatant with isolated Hb_{cyt} from the ghosts of erythrocytes containing Hb_{sm} and the remains of Hb_{cvt}. Concentration of isolated Hb in the obtained supernatant was estimated using absorbance spectroscopy. In SERS experiments, we used isolated Hb in concentrations of 10^{-6} M and $0.33*10^{-9}$ M.

To obtain erythrocyte ghosts with a different amount of Hb_{sm}, blood plasma was separated from erythrocytes by triple centrifugation (440 g, 10 min) of blood in a normal Alen's saline, pH 7.4, at 4^0C. The number of erythrocytes in the obtained erythrocyte suspension was counted in the Gorjaev's chamber and then the erythrocyte suspension was adjusted with the Alen's saline to the number of erythrocytes of 10^7 cells/numb. One volume of adjusted erythrocyte suspension was diluted with 20 volumes of ice-cold phosphate buffer (pH 7.4) and centrifugated at 4 ^{o}C and 4000 g for 40 min. Supernatant with Hb_{cyt} was removed and the precipitate with erythrocyte ghosts was resuspended in the fresh ice-cold phosphate buffer (pH 7.4) and centrifugated again at 4 ^{o}C at 4000 g for 40 min. The procedure was repeated 5 times. After that erythrocyte ghosts were concentrated in Alen's saline (pH 7.4) by centrifugation at 10000 g for 30 min. The prepared erythrocyte ghosts had pinkish color due to Hb_{sm} bound to the AE1 exchanger of the ghost membrane. The obtained erythrocyte ghosts contained only membrane-bound Hb_{sm} and had no unbound cytosolic Hb_{cyt}. The ghosts with a small amount of Hb_{sm} were prepared using the same technique with the only difference that a phosphate buffer with pH 8 replaced with the buffer having pH 7.4. The alkaline pH causes a release of most of Hb_{sm} molecules from the transmembrane protein AE1. The obtained ghosts had a white color due to the almost complete absence of Hb_{sm}. In SERS experiments we used a suspension of erythrocyte ghosts obtained by 1000-times dilution.

Table 1. Assignment of main vibration bands in Raman spectra of hemoglobin [1–4]

Frequency, cm^{-1}	Bonds in heme	Vibration mode symmetry	Factors affecting the vibrations	Hb form
1640	CaCm, CaCmH, CaCb	B1g, ν10	Redox and spin state of Fe, presence of ligand	HbO2
1620-1623	(C1C2)vinyl			HbO2, dHb
1603–1608	CaCm, CaCmH, CaCb	B1g, ν10	Redox and spin state of Fe, presence of ligand	dHb
1580–1588	CaCm, CaCmH	A2g	Spin state of Fe, diameter of heme ring	usually HbO2
1565–1566	CbC1, CbCb	B1g	Redox and spin state of Fe	HbO2
1548–1552	CaCm, CaCmH	A2g	Spin state of Fe, diameter of heme ring	usually dHb
1502	CaCm, CaCb, CaN	A1g	Redox and spin state of Fe,	HbO2
1375	CaCb, CaN,NCaN (pyrrol half-ring symmetric)	A1g, ν4	Redox state of Fe, presence of ligand	HbO2
1305	all above	ν21	Redox and spin state of Fe, presence of ligand	HbO2
1172	CaCb, CaN,NCaN (pyrrol half-ring asymmetric)	B2g, ν30	Redox state of Fe, presence of ligand	HbO2
1345	C2vinylH			HbO2
1127 754	Cb − CH3 heme breathing	B1g, ν5 B1g, ν15		HbO2 HbO2, dHb

References

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