SUPPORTING INFORMATION

Quantitative and multiplex dot-immunoassay using gap-enhanced Raman tags

Boris Khlebtsov,[a] Timophey Pylaev,[a] Vitaly Khanadeev,[a] Daniil Bratashov[b] and Nikolai Khlebtsov[a,b,*]

[a] Institute of Biochemistry and Physiology of Plants and Microorganisms,

Russian Academy of Sciences, 13 Prospekt Entuziastov, Saratov 410049, Russia

[b] Saratov National Research State University, 83 Ulitsa Astrakhanskaya, Saratov 410012, Russia

**Corresponding author, e-mail: khlebtsov@ibppm.ru*

S1. Fundamental enhancement factors (EFs) of GERTs

A typical figure of merit of the SERS response is the fundamental enhancement factor (EF), which is calculated as the ratio of SERS and normal Raman intensities normalized to the number of excited molecules.[1] In our study, the strongest SERS peaks was located at 1345 cm^{-1} for NBT, 1058 cm^{-1} for NT and 1081 cm^{-1} for AcTP. Taking into account the equal scheme for registration of SERS and normal Raman scattering, we can assume that the number of molecules is proportional to the molar concentration of molecules in solution.

$$EF = \frac{I_{SERS} / N_{SERS}}{I_{Raman} / N_{Raman}} = \frac{I_{SERS} / C_{SERS}}{I_{Raman} / C_{Raman}}.$$

For Raman scattering, this concentration was 0.1 M. To calculate the concentration of SERS active molecules, we use a simplified spherical model of our polygonal cores with diameter of 30 nm. The total surface of such particle is $S = \pi d^2 = 2830$ nm^2.

Assuming a 0.22 nm^2 footprint for each molecule,[2] the amount of Raman molecules is estimated as $S/0.22=1.28\times10^4$ per one core. Note that this is a maximal amount of SERS active molecules. Therefore, the use of this value in further calculations gives a minimal EF.

The total volume of one core is $V = \frac{\pi d^3}{6} = 1.41\times10^4$ nm^3.

The mass of one core is $m = \rho V = 2.73 \times 10^{-16}$ g

Taking into account the initial 1 mM concentration of functionalized Au core solution and further 10 times dilution during GERTs growth, the total mass concentration of Au is 17 mg/L.

The number concentration of GERTs is $17 \times 10^{-3} / 2.73 \times 10^{-16} = 6.23 \times 10^{13}$ particles/L.

The number concentration of SERS active molecules is $1.28 \times 10^{4} \times 6.23 \times 10^{13} = 8 \times 10^{17}$ molecules/L.

Finally, the molar concentration of SERS molecules in GERTs is 1.33×10^{-6} M

Table S1. The values of SERS EF and the parameters used for their calculations

	Concentration, M	Intensity, counts	EF
NBT 0.1M (1345 cm^{-1})	0.1	630	N/D
NT 0.1M (1058 cm^{-1})	0.1	260	N/D
AcTP 0.1M (1081 cm^{-1})	0.1	220	N/D
GERT@NBT	1.33×10^{-6} M	4.62×10^{4}	5.5×10^{6}
GERT@NT	1.33×10^{-6} M	1.67×10^{4}	4.8×10^{6}
GERT@AcTP	1.33×10^{-6} M	1.42×10^{4}	4.9×10^{6}

2. **Multiplexed detection of R-IgG, H-IgG, and Ch-IgG analytes by Raman intensity mapping**

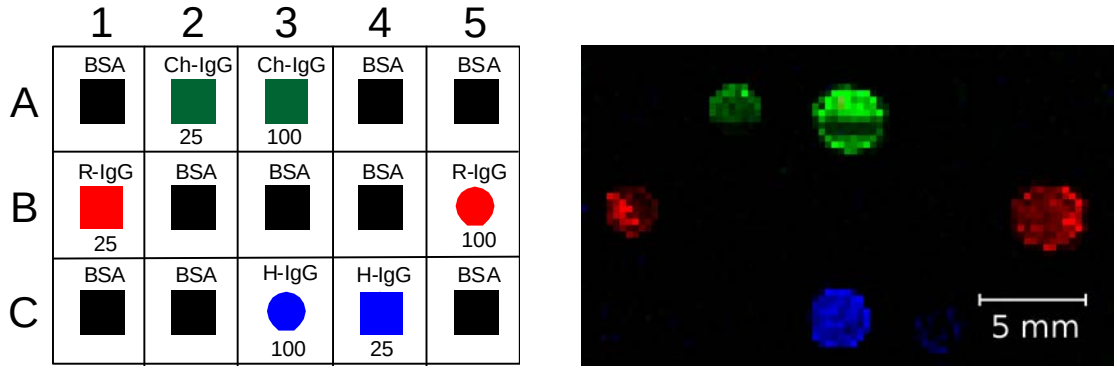

Fig. S1. Multiplexed detection of R-IgG, H-IgG, and Ch-IgG analytes by Raman intensity mapping of membrane shown in Fig. 4b of the main text. Note the difference in colour intensities corresponding to the difference in analyte concentrations (25 and 100 μg/mL).

References

1. Le Ru, E. C.; Blackie, E.; Meyer, M.; Etchegoin, P. G. *J. Phys. Chem. C*, 2007, **111,** 13794−13803.

2. N. Gandra and S. Singamaneni, *Adv. Mater.*, 2013, **25**, 1022-1027.