

ABO blood groups antigen-antibody interactions studied by SERS spectroscopy: towards blood typing

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1. SERS properties of silver-gold bimetallic surface. Enhancement factor calculation.

In order to examine the SERS activity of the silver-gold bimetallic surface, we calculated the enhancement factor and *p*-MBA (*p*- mercaptobenzoic acid) was chosen as a standard analyte. The surface enhancement factor (EF) for *p*-MBA was calculated according to the following equation:

$$EF = \frac{I_{SERS}N_{NR}}{I_{NR}N_{SERS}} \quad (1)$$

where N_{SERS} and N_{NR} refer to the number of molecules adsorbed on the SERS probe within the laser spot area and the number of molecules probed by regular Raman spectroscopy, respectively. I_{SERS} and I_{NR} correspond to the SERS intensity of *p*-MBA on the modified surface and to the normal Raman scattering intensity of *p*-MBA in the bulk. I_{NR} and I_{SERS} were measured at 1079 cm⁻¹. For the target *p*-MBA molecule in the measured sampling area (5 μm in diameter), N_{SERS} was calculated to be about 4.2×10^9 . Taking into account the laser

spot diameter (5 μm), the penetration depth (about 2 μm), and the effective illuminated volume of our setup ($2 \times 10^3 \mu\text{m}^3$), N_{NR} reaches the value of 8.1×10^{12} . From these data of the relative intensity and the number of molecules sampled from the regular Raman and SERS measurements, the enhancement factor was calculated to be about 2.3×10^6 . The achieved level of enhancement makes this method of SERS platform fabrication a promising strategy for practical SERS applications and was applied for blood analysis.

2. The ABO blood groups antigen-antibody interactions studies.

Below are gathered the results which evidently show that differentiating the ABO blood groups may be performed only with the antigen-antibody interaction study.

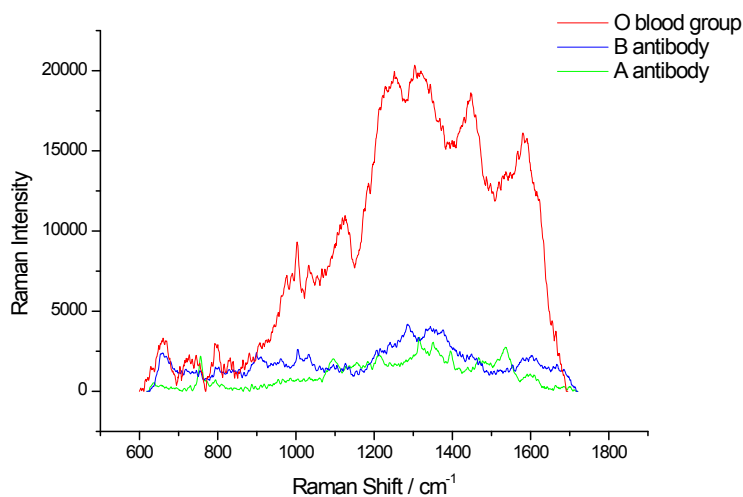
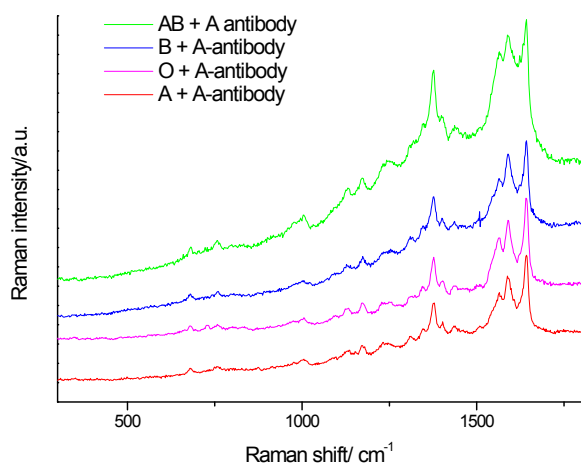


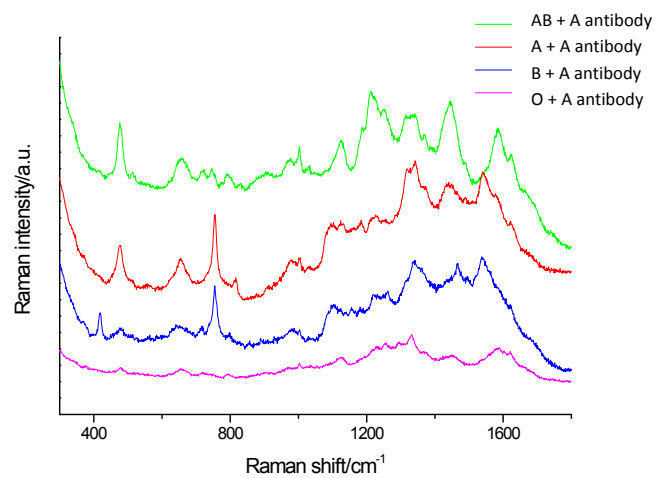
Fig. S1. SERS spectra of O blood group and A and B antibodies. Comparison of intensities.

a) ABO system with A antibody

514 nm

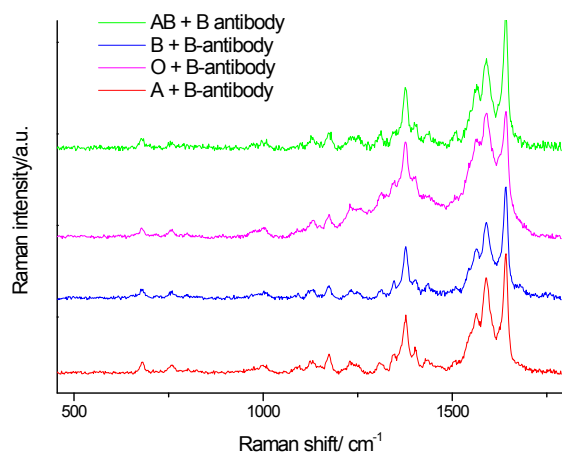


785 nm



b) ABO system with B antibody

514 nm



785 nm

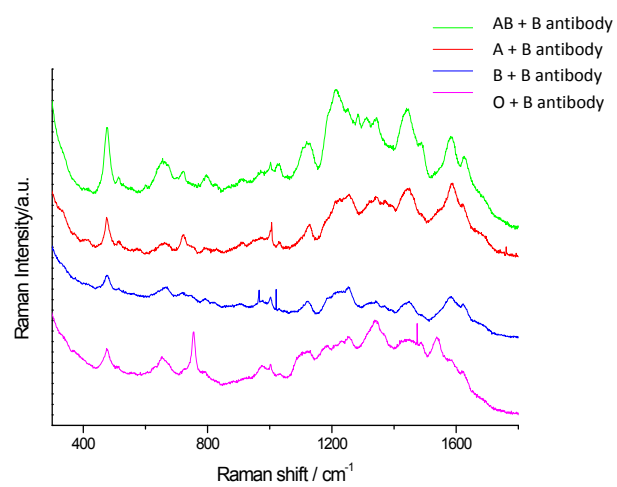


Fig. 2S. SERS spectra of ABO blood group with A antibody (a) and B antibody (b) recorded with 514 nm and 785 nm.

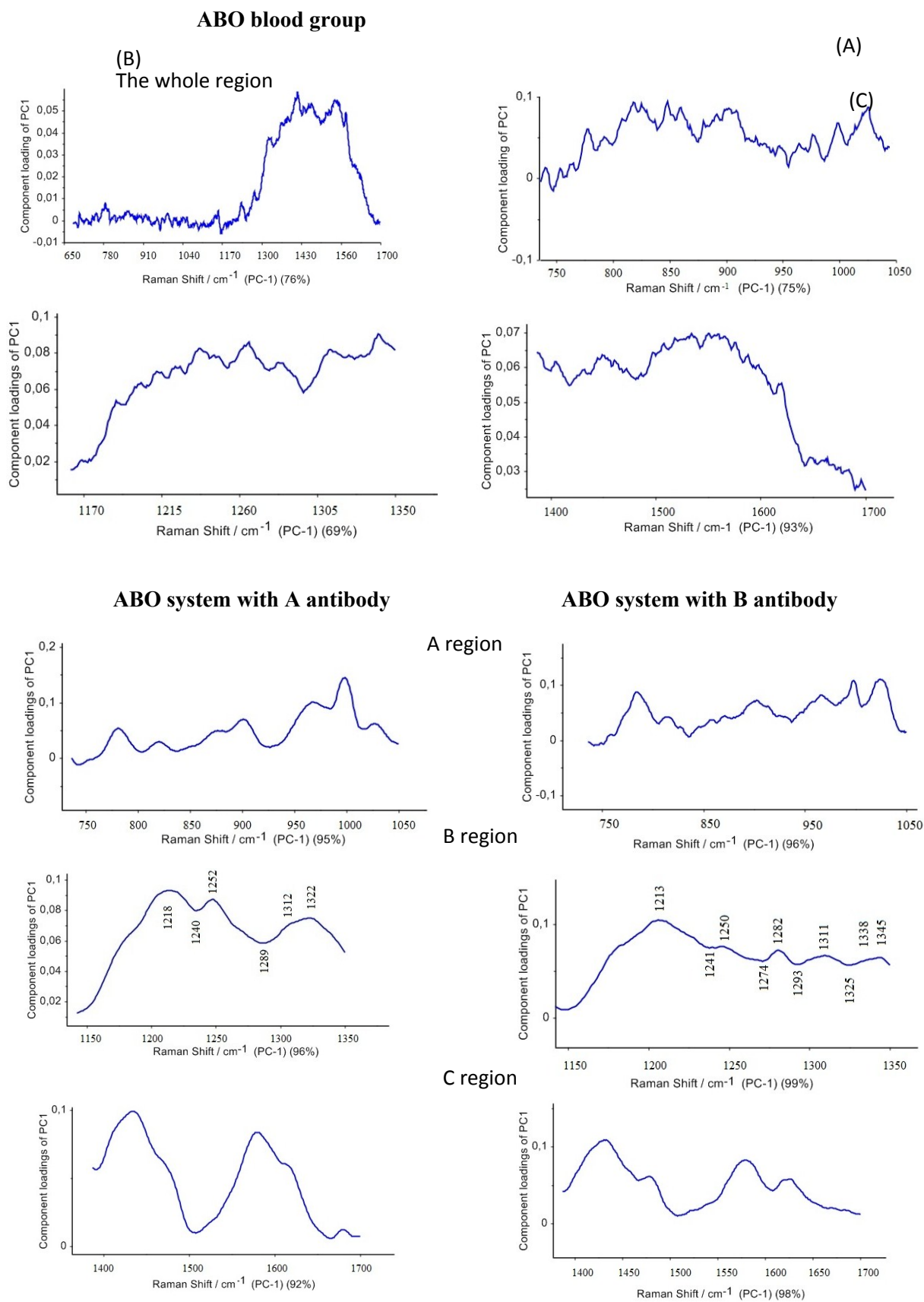


Fig. 3S. PCA1 loadings obtained for all ABO blood group together and their mixtures with A or B antibody. In brackets (A, B and C – according to Figure 5, main text), calculated loadings for selected ranges of Raman spectra.

3. The calculation of the reproducibility of SERS spectra.

The reproducibility analysis of recorded the SERS spectra for each ABO system with A and B antibody respectively, were processed with a Savitzky-Golay second derivative method. Correlation coefficients between all non-identical spectral pairs ($i \neq j$) in the same date set were determined from the data according to the formula:

$$P_{i,j} = \frac{\sum_{k=1}^W (I_i(k) - \bar{I}_i) (I_j(k) - \bar{I}_j)}{\sigma_i \sigma_j} \quad [1]$$

where i, j were the indexes of the spectra in the data matrix, k was the wave number index of the individual spectra, I was the spectral intensity, W was the spectral range, and σ_i was the standard deviation of the spectrum.^[1] Once the correlation coefficients $P_{i,j}$ were calculated, Γ , the average of the off-diagonal correlation coefficients, was determined:

$$\Gamma \equiv \frac{2 \sum_{i=1}^N \sum_{j=i+1}^N P_{ij}}{N(N-1)} \quad [2]$$

Γ varied between 0 and 1, where 1 was the case of identical spectra and 0 the case of completely uncorrelated spectra. Γ , as defined in Equation 2, was used to evaluate the reproducibility of the recorded spectra. In Fig. S4 are gathered the obtained results. The calculated average spectral correlation coefficients are gathered in the Table 1S.

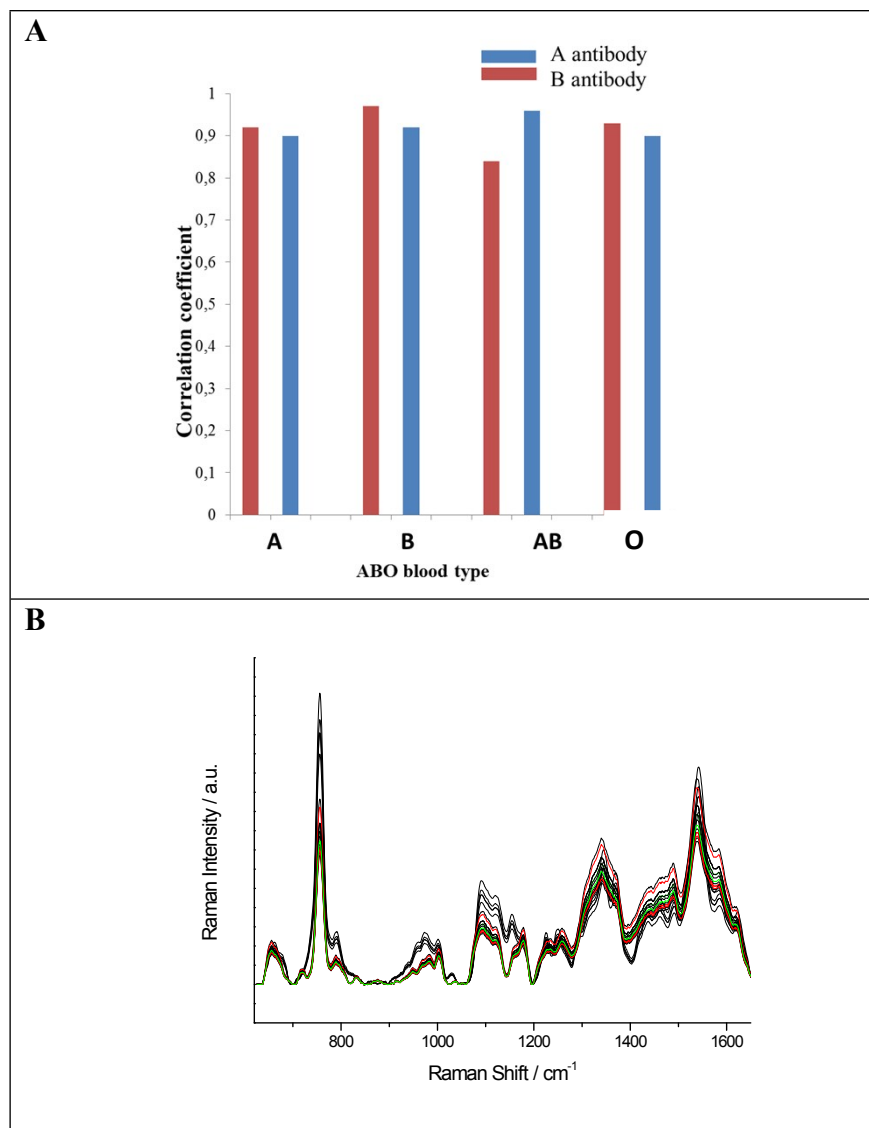


Fig. 4S. (A) The correlation coefficients of the SERS spectra of A, B, O and AB systems with A or B antibody. (B) The example of reproducibility of 20 SERS spectra for A blood group with A antibody (20 spectra recorded from different points at one sample).

Table 1S. The calculated average spectral correlation coefficients for each ABO systems with A and B antibody respectively.

Blood system	Γ (correlation coefficient) calculated for	
	A antibody	B antibody
A	0.92	0.90
B	0.97	0.92
O	0.84	0.96
AB	0.93	0.85

3. Validation of the proposed method.

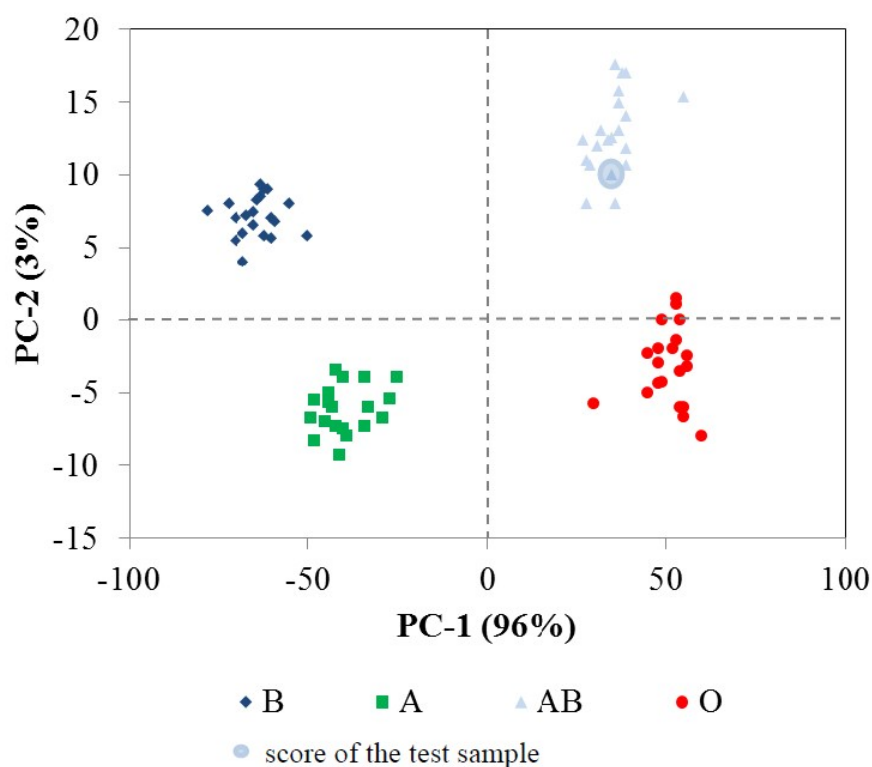


Fig. 5S The PC-1 versus PC-2 scores obtained from PCA analysis of A- antibody mixed with RBCs of all ABO system (based on 80 SERS spectra from 4 patients). The test sample (additional external blood sample with known AB group) was introduced into optimized PCA model for validation of proposed method for blood typing.

As can be seen from Fig. 5S the test sample (AB blood group) gives the score located in the cluster of other PC scores of AB blood system. This results present the potential of SERS combined with PCA analysis for differentiation of ABO system.

[1] A. Barhoumi, D. Zhang, F. Tam, *J.Am.Chem.Soc.* **2008**; *130*, 5523.