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Modification of Ag SERS-active surface to promote charged analytes adsorption

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Abstract

This work aims at the impact of the electrostatic interaction between analyte molecules and silver nanoparticles (Ag NPs) on the surface enhanced Raman scattering (SERS) performance. For this, we fabricated nanostructured plasmonic films by immobilization of Ag NPs on glass plates and functionalized them by a set of differently charged hydrophilic thiols (sodium 2-mercaptoethyl sulfonate, mercaptopropionic acid, 2-mercaptoethanol, 2-(dimethylamino) ethanethiol hydrochloride and thiocholine) to vary the surface charge of the SERS-substrate. We used two oppositely charged porphyrins, cationic Cu(II)-tetrakis(4-N-methylpyridyl) porphine (CuTMpyP4) and anionic Cu(II)-5,10,15,20-tetrakis(4-sulphonatophenyl) porphine (CuTSPP4), with equal charge value and similar structure as model analytes to probe SERS signal. Our results indicate that the SERS spectrum intensity strongly, up to complete signal disappearance, correlates with the substrate's surface charge that tends to be negative. Using the data obtained and our model SERS-system, we analyzed modification of Ag

surface by different reagents (lithium chloride, polyethyleneimine, polyhexamethylene guanidine and multicharged metal ions). Finally, all those surface modifications were tested using a negatively charged oligonucleotide labeled with Black Hole Quencher (BHQ1) dye. Only addition of copper ions into the analyte solution allowed to get a good SERS signal. Considering strong interaction of copper ions with the DNA molecule, we suppose that the analyte charge inversion played the key role in that case, instead of the recharging of the substrate surface. Analyte recharging could be a promising way to get intensive SERS spectra of negatively charged molecules on Ag SERS-active supports.

Keywords: Surface-enhanced Raman spectroscopy, silver nanoparticles, substrate modification, electrostatic interaction, oligonucleotides, porphyrin

Introduction

Surface-enhanced Raman scattering (SERS) with advantages of extreme sensitivity, high selectivity, and non-destructive nature has demonstrated a great potential for express detection of chemicals in different samples [1]. It became popular in the scientific community during the last decades due to great prospects for the practical solutions of, particularly, analytical problems [2]. However, despite of promising potential possibilities, it turned out that a lot of practical, theoretical, and even technical tasks should be solved for real practical applications of the method [2-4].

The Raman signal surface enhancement implements using so-called SERS-active substrates that are mainly inorganic or hybrid nanostructured materials. Significant attention has been devoted to the development of metallic NP arrays formation methods with controllable parameters such as size, shape, inter-particle distance, and ordering degree [5-12] with the main focus concentrated on plasmonic structures with high ”hot spots” density. Due to the progress in nanotechnology, significant amount of highly sensitive SERS substrates have been synthesized [1, 13].

The SERS substrate design ordinarily aims at maximizing the plasmonic effect of the Raman enhancement. There are two generally recognized mechanisms responsible for the SERS enhancement: electromagnetic enhancement (EM) and chemical enhancement (CE) [14,15]. The basic mechanism is EM produced by LSPRs on the plasmonic metal surface [16]. The CE is at least two orders of magnitude weaker than EM. The CE mechanism is supposed to be caused by a charge transfer between the plasmonic surface and the chemically adsorbed analyte molecules that introduces new states in the electronic structure of the metal-adsorbate complex leading to an increase in the Raman scattering cross-section of the analyte [17]. Thus, if the charge transfer states are in the resonance with the incident light, the SERS signal could be further amplified by the resonance effect.

Since the LSPR-enhanced electromagnetic field decays exponentially with the distance from the metal surface, the analyte molecules should be located near the surface of the SERS substrate to achieve maximum enhancement. However, close proximity is not optimal because of possible quantum tunneling effects [2]. The SERS in the "hot spots" suffers from those undesired effects even more, because the analyte molecules have to be localized in a small volume in gaps between NPs [4]. Thus, a principal challenge in using SERS for sensitive but safe detection is to localize the molecules of interest at the plasmonic surface but at a proper distance. It is a common practice, that the SERS-effectivity of a SERS-active substrate is usually demonstrated by using analytes that are strongly adsorbed at the plasmonic metal surfaces and located at the "hot spots". In fact, detection of analytes that are not interacting (or adsorbing) with plasmonic surface, remains an important practical task. This problem significantly hampers a wider practical application of SERS because even optimally fabricated SERS-substrates lose their effectiveness when the analyte molecules cannot access to the "hot spots".

To address this problem, various techniques have been developed to capture non-adsorbing molecules on the SERS-active surface [3, 18, 19, 20]. They are mainly based on

functionalization of plasmonic nanostructures for the analyte targeted capture. Another possibility related to the chemical modification of the analyte molecule, remains much less developed. Recently, chemical modifications of the plasmonic surface improved the SERS performance in different applications, such as environmental monitoring, food-handling or biomedicine [21-25]. Usually, molecules having a thiol or amine group in their structure are used for the plasmonic surface functionalization. This leads to the stable binding to metal surfaces, which results in high SERS signals. Simple electrostatic attraction can be very effective [26], for example, when conventional citrate- or hydroxylamine-reduced colloids of negatively charged particles are used to detect positively charged analytes [27]. Additionally, SERS substrates modified with molecules bearing alkyne or nitrile functional groups were used because Raman frequencies of alkyne and nitrile bonds were located outside the region of the SERS spectrum biological molecules [28, 29].

Among various plasmonic (Au, Ag, Cu and Al) materials, gold is the most convenient to SERS-based biological sensors due to its high chemical stability and quite strong localized urface plasmon resonance (LSPR). Silver SERS substrates provide the strongest Raman enhancement for the same structure [8] and therefore are promising for wide practical application. At the same time, Ag is much more chemically active than Au. Consequently, the use of the corresponding Ag-based SERS-active systems is complicated by additional chemical processes. The most exciting example is the influence of halide ions on the SERS signal. It is due to the high binding energy of Ag with halides that is also responsible for the low solubility of the corresponding salts in water. The SERS activation of cationic analytes was observed in a number of studies after treatment of nanoparticles with halide ions [30-33]. Unusual effect of lithium chloride on the SERS intensity signal was reported as well [32]. On the other hand, the almost complete absence of the SERS signal was reported for nanomaterials, which were prepared in the absence of halides [31].

It should be noted that the role of halide ions in the SERS activation of Ag based substrates is not yet fully clarified. A possible explanation could be based on their effect on the electrostatic interaction between analyte molecules and the Ag NPs surface. Literature analysis reveals that many authors use cationic organic dyes as SERS-probing analytes [18,34]. In contrast, practically interesting biological molecules are mostly negatively charged. In 2015, the authors of [18] pointed out a possibility to prepare positively charged Ag NPs to analyze anionic analytes which are hardly detectable by the Ag NPs prepared by common protocols. They used thiocholine to create strong positive charge on the Ag NPs surface. However, the solution of the charge problem was not so simple because the citrate ions which are present in many common protocols caused aggregation of the resulting positively charged Ag NPs. Nevertheless, the approach proposed permitted successful analysis of a number of negatively charged analytes. Silver NPs treated with polyethyleneimine, spermine, or spermidine were successfully used for detection of negatively charged oligonucleotides [19]. Finally, recent work [20] demonstrates detection of anionic analytes by addition of multi-charged metal cations. The above-mentioned papers devoted to the chemical SERS activation of silver-based substrates, with exception of [32, 33] described colloidal NPs study. The work [20] reports the absence of the NPs aggregation in the presence of multi-charged metal ions. Considering strong tendency of multi-charged ions to destabilize colloidal systems even at low concentrations, this observation looks surprising. It should be noted that it is difficult to control aggregation of NPs in colloidal substrates, which can occur even in absence of any dopants. However, SERS signal from the aggregates due to creation of “hot spots” can be orders of magnitude higher than the signal from the non-aggregated NPs [2,4]. Thus, even a small amount of the aggregates that is not visible in the absorption spectra of the solution can lead to a significant increase of the SERS intensity, obscuring effect of functionalization of the NPs surface. In contrast, solid-based substrates have

improved reproducibility of the SERS enhancement and provide exact location of the target molecules that makes possible to exploit different techniques of functionalization.

To avoid different effects related to NPs aggregation, in our study we used Ag NPs immobilized by adsorption as a convenient and reproducible SERS substrate for investigation of the Ag surface chemical treatment with different reagents. To study electrostatic interaction with an analyte and exclude factors caused by complex specific interactions with Ag surface, we used a set of organic thiols with differently charged functional groups and similar linker length. Additionally, we checked previously reported approaches for the Ag surface modification (polymers, lithium chloride, multi-charged metal ions) to improve SERS signal of substrates.

Results and Discussion

Characterization of silver nanostructures. We conducted Ag NPs synthesis according to a protocol allowing formation of NPs with the size (30-35 nm) appropriate for the SERS investigation and for obtaining relatively high NPs concentration (32 μg/ml) sufficient for good immobilization kinetics. The NPs size and shape were analyzed from the SEM and TEM images. To achieve better resolution of SEM, we utilized conductive silicon support and excluded metal coating of the sample; Ag NPs were placed to the silicon surface by adsorptive immobilization method to avoid aggregation during solvent evaporation and enhanced particles recognition as a result.

Figure 1 demonstrates SEM of the NPs as well as the corresponding size distribution histogram. Similar TEM image (Figure S1) shows fewer nanoparticles and is therefore less reliable. Both methods give NPs diameter in the interval of 30-35 nm. According to the SEM image, the NPs mostly have spherical shape and 33 nm average diameter. At the same time, as SEM and TEM images demonstrate similar size distribution. So, we conclude that no NPs size selection occurs during their adsorptive immobilization. Silver NPs absorption spectrum (Figure S2) is in good agreement with the electron microscopy and literature [35].

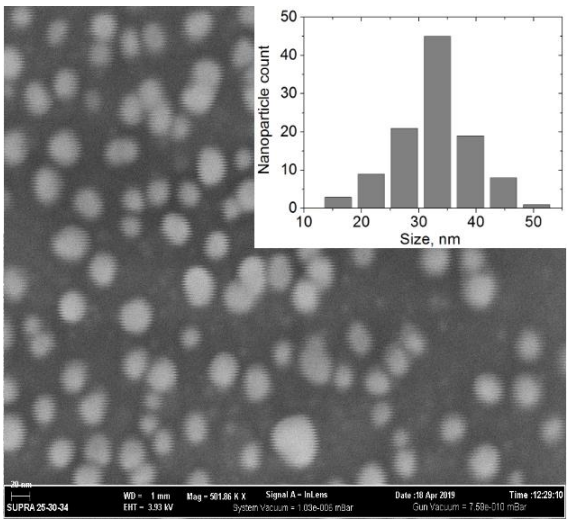

Figure 1. SEM images of Ag NPs and corresponding size distribution histogram. To get SEM image, Ag NPs were deposited onto PEI-modified silicon conductive support by the adsorptive immobilization method.

Immobilization of Ag NPs on glass surface. Utilizing glass slides for SERS substrate preparation permitted us to use siloxane chemistry to modify surface and to control immobilization process by the absorption spectra. Glass was also advantageous to minimize additional effects related to SERS enhancement due to the energy transfer to the support, which would be possible if conductive or semi-conductive materials were used [36]. Ag NPs immobilization from sodium citrate solution was possible due to poor citrate ions adsorption by Ag surface leading to high immobilization efficiency. Sodium citrate concentration (2.5 mM) was adjusted to achieve maximal NPs surface filling and, at the same time, to avoid NPs aggregation caused by high salt concentration.

Figure 2 demonstrates absorption spectra of the PEI-modified glass support (slide) depending on the time of its treatment by Ag NPs. The maximum absorption value stabilizes after approximately 12 h with a shift from 412 nm to 427 nm. We suppose that the absorption maximum red-shift is due to the plasmon interactions of the closely packed NPs.

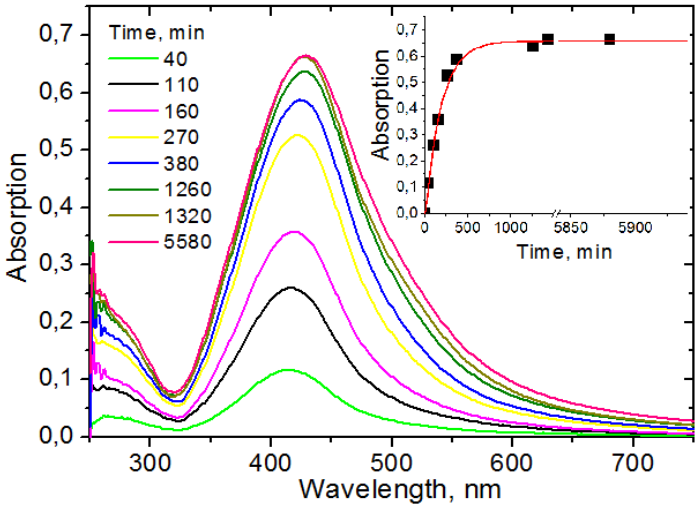

Figure 2. Silver NPs immobilization kinetics on glass substrate. Since glass slides are modified on both sides, the spectra reveal absorption of two NPs layers.

In order to investigate surface coverage of the glass SERS substrates, we utilized SEM image of the PEI-modified silicon support with analogous NPs treatment supposing equal packing density (Figure 3). The surface coverage degree obtained was 58% that is 74% of the theoretical value for spherical particles with equal sizes and corresponds to the highest reported values for this method [10, 12, 39]. Moreover, the SEM image demonstrates many inter-particle contacts, which are potential “hot spots” in SERS analysis.

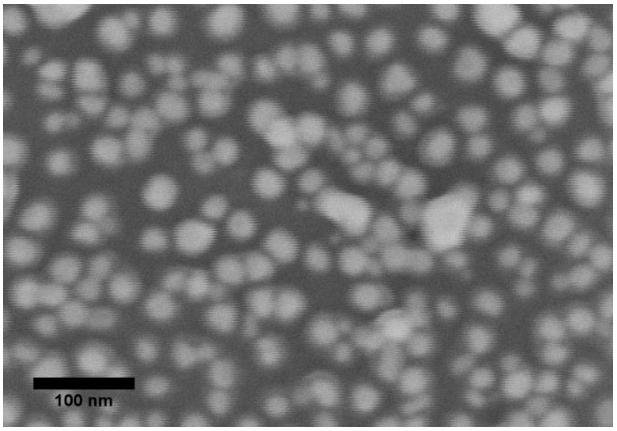

Figure 3. SEM image of Ag NPs immobilized on silicon surface by optimized procedure. The surface coverage degree is 58%.

Electrostatic effect on SERS signal for Ag NPs modified with thiols. Despite of the high NPs packing density, about 42% of the support surface remains unoccupied, thus providing space for an analyte localization that would occur in the case of its poor interaction with the NPs surface. Besides, a rough theoretical assumption of the PEI modifying layer thickness performed using its molecular weight (25 kDa) and its branched structure gives the value below 10 nm. Taking into account the NPs diameter, we conclude that the major part of their surface would remain uncovered by the polymer layer in the SERS substrate plasmonic film. AFM images (Figure S3) confirm our conclusion. Topology map shows that the NPs are exposed out of the polymer layer. Adhesion image proves that the surface properties of the immobilized Ag NPs strongly differ from the glass ones, and the polymer does not cover them. Weak citrate interaction with Ag surface permits NPs treatment with different reagents and study resulting surface effects influence on the SERS intensity. Thus, prepared SERS substrates function as a convenient instrument for surface affinity investigation at different surface modifications and analyte types.

To exclude uncontrollable interactions of the analytes with Ag surface, we applied in our study a set of organic thiols with the same linker length but differently charged hydrophilic functional groups. Thiol functionality guaranties strong binding to the surface and dense enough molecule packing to make the surface properties determined by the introduced functional group. We used short ethylene linker and hydrophilic functional groups to avoid lipophilic interactions. Two hydrophilic porphyrins with similar structures, equal charge value and opposite charge (CuTMpyP4 and CuTSPP4) were used as model analytes. Structures of the modifying molecules and the analytes are depicted on Figure 4.

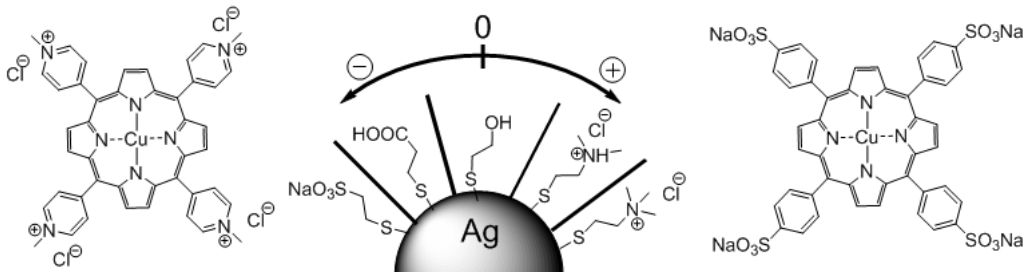

Figure 4. Schematic representation of immobilized Ag NPs surface modification by thiols bearing differently charged functional groups and oppositely charged model analytes CuTMpyP4 and CuTSPP4.

Initially prepared plasmonic nanostructures demonstrated rather intensive SERS signal for CuTMpyP4 bearing positive charge (Figure 5: 6(a)), however, did not give any spectrum for the negatively charged porphyrin CuTSPP4 (Figure 5: 6(b)). Treatment of Ag NPs with sodium mercaptoethyl sulfonate did not result in significant change of the SERS intensity for the both porphyrins (Figure 5: 5(a,b)).

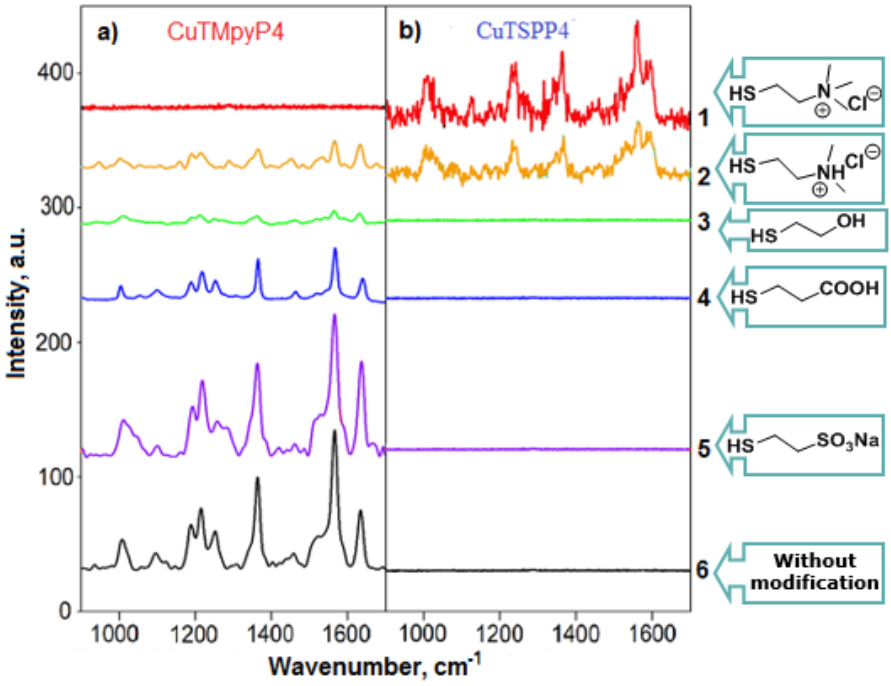

Figure 5. SERS spectra of CuTMpyP4 (a) and CuTSPP4 (b) drop-casted from 10^{-6} M solution onto the substrate modified with thiocholine (1), 2-(dimethylamino)ethanethiol hydrochloride

(2), 2-mercaptoethanol (3), mercaptopropionic acid (4), sodium mercaptoethyl sulfonate (5), and unmodified plasmonic surface (6).

These data are in line with the initial negative charge of the NPs surface. High affinity of thiol groups to Ag surface suggests that chloride counter ions of CuTMpyP4 do not significantly influence the SERS signal. The use of mercaptopropionic acid for the modification leads to CuTMpyP4 SERS intensity decrease (Figure 5: 4(a,b)) with no SERS signal of CuTSPP4, presumably, because of the lowering of the negative charge of the Ag surface due to several orders of magnitude lower acidity constant of the introduced carboxylic groups compared to sulfate ones. Using neutrally charged mercaptoethanol for the surface modification led to almost full absence of both porphyrins SERS spectra (Figure 5: 3(a,b)). In this case, the Ag surface became uncharged and the analyte molecules were localized only on the glass support surface.

Modification of Ag NPs with 2-(dimethylamino)ethanethiol hydrochloride results in about fourfold SERS intensity decrease for the cationic analyte. A weak CuTSPP4 spectrum is registered at this modification (Figure 5: 2(a,b)). Such result witnesses sufficient charge lowering due to introduction of weakly basic amine groups to the Ag surface. The presence of SERS signal of the both analytes can be explained by additional donor-acceptor interaction of Cu atoms with electronic pair of the amine groups. Another explanation is a zwitter-ionic structure formation on the NPs surface. Thiocholine surface modification causes complete CuTMpyP4 SERS signal disappearance and significant spectrum intensity increase for CuTSPP4 (Figure 5: 1(a,b)). We explain this by positive charging of the Ag surface. However, SERS signal of CuTSPP4 remains much weaker in that case than that for CuTMpyP4 with negatively charged surface modification. It seems not to be caused by different adsorption maxima of the analytes because they have very similar optical properties [40]. We suggest that some negatively charged centers are still present at the NPs surface after thiocholine modification such as adsorbed chloride thiocholine counter ions, for example, which contribute to the resulting surface charge

and make it weaker. The experimental data obtained support previous reports about great importance of the electrostatic interaction for charged analytes in the SERS analysis [18,20] and once again points out the problem of SERS substrates with high surface charge.

Other methods of silver surface modification in the context of electrostatic interaction with analyte. We also made experiments on other methods of plasmonic surface modification using our model SERS system, and compared the results with those reported in literature, *e.g.*, coating with polymers [7,19] or different inorganic reagents [31,32,20]. Figure 6 shows corresponding modification schemes. Treatment of the plasmonic surface with lithium chloride led to threefold increase of SERS intensity for the positively charged porphyrin (Figure 7, 3(a)) whereas any spectrum of anionic CuTSPP4 were not observed. The result supports an earlier report about SERS activation by LiCl [32] and contradicts to the authors' statement that the effect is not surface-charge dependent. We suppose that the advantage of LiCl compared to other basic metal halides is due to poorly soluble $LiAgCl_2$ formed at the Ag surface, which, according to our results, enhances surface negative charge. However, the complex substance has crystal structure and can form differently terminated regions on the surface, which can adsorb different molecules giving rise to their SERS signal. High specific affinity of Ag to the analytes could also promote SERS activity enhancement for the anionic analytes described in [32].

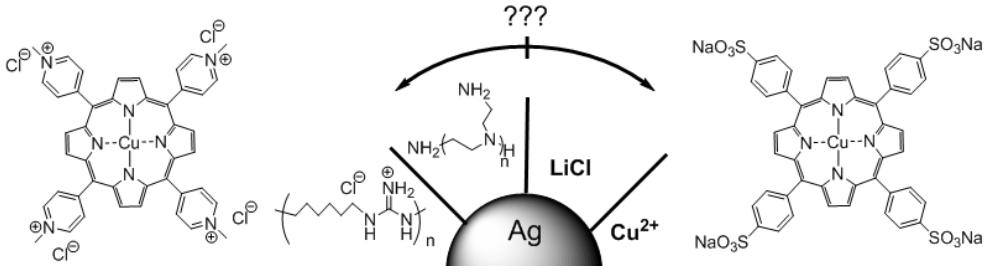

Figure 6. Schematic illustration of Ag surface modifications by PHMG, PEI, LiCl and Cu^{2+} using oppositely charged model analytes CuTMpyP4 and CuTSPP4.

We also studied SERS activity increase for negatively-charged analytes by means of multiply-charged cations [20]. For this purpose, copper cations were selected since they are already present in the structure of the both analyte molecules and thus their chemical transformation can be excluded.

Treatment of the NPs surface with Cu^{2+} ions resulted in about twofold increase of the CuTMpyP4 SERS intensity. Under the same conditions, CuTSPP4 SERS spectrum was absent (Figure 7: 5(a,b)). We suggest that Cu^{2+} ions are weakly adsorbed at the Ag surface and were mainly removed during the washing stage. Copper complexation with PEI and positive charging of the glass surface is expected in this case. This could also explain the increase of the cationic analyte SERS. Furthermore, we checked the effect of the Cu^{2+} cations addition to the analyte on the SERS intensity. CuTMpyP4 SERS intensity becomes five times lower in the presence of Cu^{2+} ions (Figure 7: 4(a,b)). At the same time, weak SERS signal of CuTSPP4 was detectable. These observations suggest that NPs surface charge remains negative, and Cu^{2+} ions do not create a strong positive charge on the Ag surface. We suppose that CuTSPP4 detection in this case is related just to the shielding of the analyte molecules by Cu^{2+} ions.

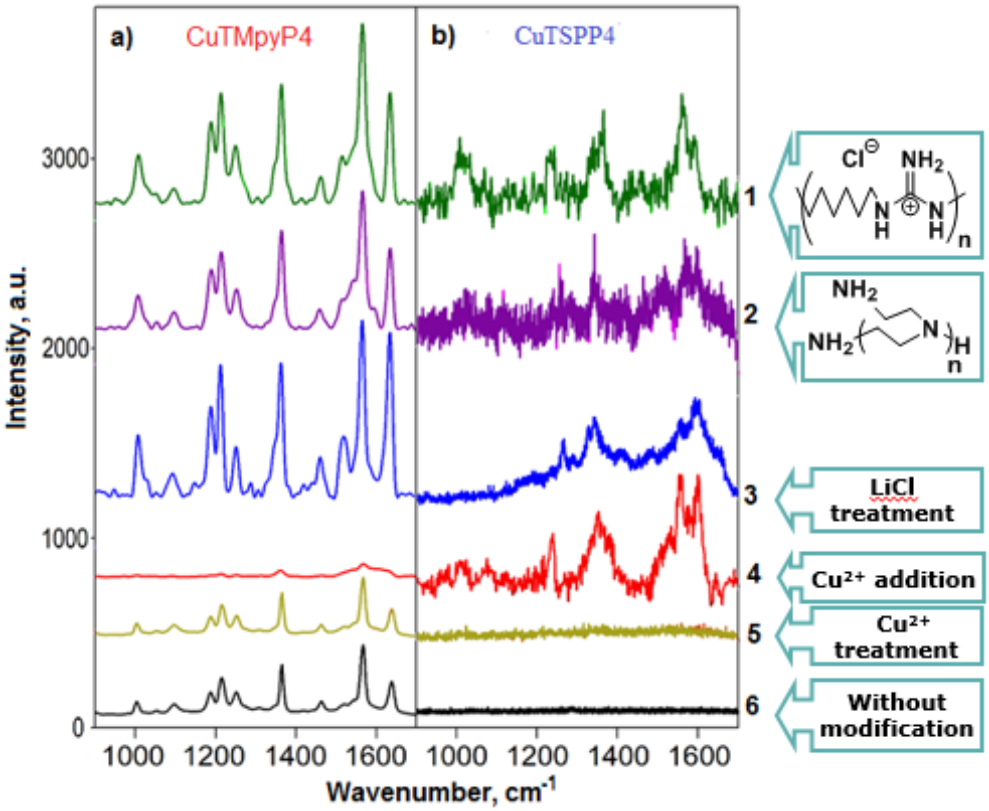

Figure 7. SERS spectra of CuTMpyP4 (a) and CuTSPP4 (b) drop-casted from 10^{-6} M solution onto the SERS substrate modified with PHMG (1), PEI (2), LiCl (3), Cu^{2+} (added into analyte solution) (4), Cu^{2+} (surface treatment) (5) and unmodified plasmonic surface (6).

Polyethylenimine and polyhexamethylene guanidine were used as basic polymer modifiers to create positive charge on Ag surface (Figure 7: 1(a,b) and 2(a,b)). Both of them were described as effective Ag NPs stabilizers which lead to NPs with positive zeta-potential [7,19]. We found that Ag NPs treatment with those polymers resulted in significant increase of the CuTMpyP4 SERS signal: threefold for PEI, and fourfold for PHMG. A weak CuTSPP4 spectrum was only observable in the case of PHMG caused by the polymer molecules partial desorption from the surface by washing. Remaining polymer molecules are not able to generate strong positive charge at the surface but they improve adsorption of the analyte molecules due to hydrogen bonding as well as by donor-acceptor and lipophilic interactions.

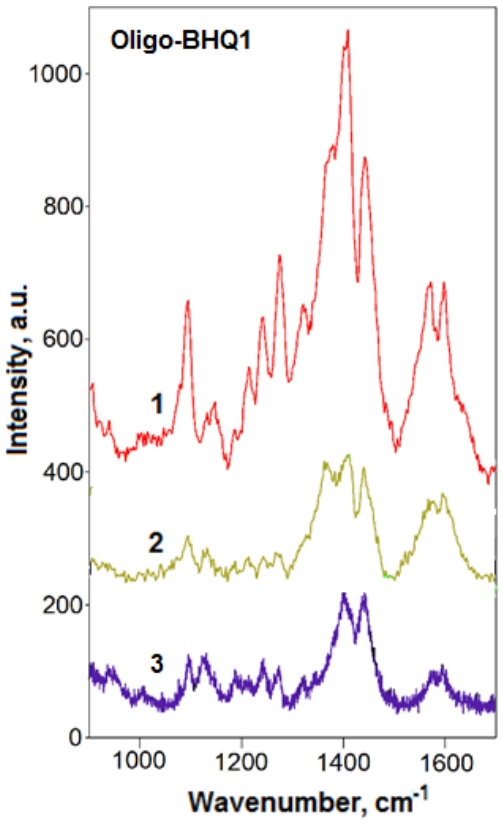

Figure 8. SERS spectra of BHQ1-labeled oligonucleotide drop-casted from 10^{-5} M solution onto substrate modified with Cu^{2+} (added into the analyte solution) (1), Cu^{2+} (surface treatment) (2); Raman spectrum of BHQ1 in solution of Ag NPs (3).

SERS analysis of dye-labeled oligonucleotide. Biomolecules like nucleotides and nucleic acids are of great interest for practical SERS applications. Oligonucleotides are the most suitable among biomolecules for the electrostatic interaction study because of their strong negative charge. Recently, we showed a crucial role of electrostatic interaction of oligonucleotides with NPs surface for bioconjugation [42]. Thus, oligonucleotide SERS spectrum can indicate about a positive charge for the SERS substrate surface. Oligonucleotide was labeled with a dye (BHQ1) for better spectrum recognition due to exclusion of characteristic peaks with background fluorescence, which is possible in the case of luminescent dyes.

Unmodified SERS substrate did not show any SERS signal of the oligonucleotide (Figure 8). Based on our previous experiments on Ag surface modification with thiols, we achieved the

best results for the negatively charged analytes by using thiocholine as a modifier. The method allowed registering good enough CuTSPP4 spectrum. We applied this approach to obtain the oligonucleotide SERS spectrum, but failed. It supports our idea about negatively charged centers at the Ag surface that decrease resulting positive charge of the thiocholine modified surface. Consequently, PEI-modified glass surface becomes more appropriate for the competitive oligonucleotide localization.

From a range of different modifications, only treatment with copper ions appeared to be successful (Figure 8: 1,2). According to earlier investigation [41], the Cu^{2+} ions are able to bind effectively both as phosphate groups and nucleobases in the oligonucleotide molecules to provide the charge inversion. This is a key factor for the Cu^{2+}-modified oligonucleotide SERS detection. The spectrum detection for Cu^{2+} ions treatment, followed by washing (that was not working for CuTSPP4), supports that and can be explained by low Cu^{2+} ions concentration created by residual PEI-copper complex. Additionally it was confitrmed in experiments with Mg^{2+} ions instead of Cu^{2+} ones, which are much less capable of complexation with nucleobases. Moreover, Mg^{2+} cations have been shown [41] to have the lowest among binary charged ions affinity to DNA molecule (Mg^{2+}< Co^{2+}< Ni^{2+}< Mn^{2+}< Zn^{2+}< Cd^{2+}< Cu^{2+}) and in contrast to the other ions they do not induce rewinding of the double helix. Addition of the Mg^{2+} ions to the analyte did not result in SERS spectrum registration of the oligonucleotide as well as CuTSPP4.

Thus, our results emphasize the importance of the SERS substrate surface charge for successful SERS detection and point out another possible way to manage it: the analyte charge inversion by interaction with positively charged metal cations. This way requires selection of the metal cations with high affinity to the analyte molecules. As demonstrated in [41], this selection is not always easily predictable from the basic knowledge of their complexation.

Conclusion

Effects of electrostatic interaction between analyte molecules and plasmonic surfaces on the SERS enhancement was investigated by using Ag NPs immobilized on the glass support. Two oppositely charged porphyrins with similar structures and the same formal charge (cationic CuTMpyP4 and anionic CuTSPP4) were used as model analytes. The surface charge of Ag NPs was adjusted by hydrophilic thiols. In the case of cationic CuTMpyP4, shift from initially negative Ag surface charge to the positive one leads to SERS intensity decrease up to complete spectrum disappearance. On the other hand, positive charging of Ag surface permits CuTSPP4 spectrum registration, which is prevented for the negative charge surface modifications. However, it remains relatively weak revealing Ag surface have tendency to be negatively charged. Thus, generation of the strong positive charge on the Ag surface is important for successful SERS detection of negatively charged analytes.

Previously described approaches for Ag surface modification such as PEI, PHMG, LiCl and multi charged metal ions led mainly to the SERS enhancement of CuTMpyP4: threefold for PEI, fourfold for PHMG and LiCl. Only addition of Cu2+ ions to the analyte permitted satisfactory registration of the CuTSPP4 SERS spectrum. Nevertheless, under similar conditions, the SERS signal of CuTMpyP4 was still present which means that the surface charge was not inverted by the addition of Cu^{2+} ions. At the same time, this was the only method that allowed to get SERS spectrum of the negatively charged dye-labeled oligonucleotide which was shown to interact strongly with Cu^{2+} ions. We suppose that the charge inversion of the analyte, is the key factor in this case. Thus, our results shift the focus from the Ag surface modification to the charge inversion of the analyte molecules, showing its perspective use for negatively charged analytes.

Experimental

Materials. Dye labeled oligonucleotide 5’-CCTGCGATCTCTCTATCCAG-[BHQ1]-3’ was purchased from Primetech ALC (Minsk, Belarus). Cu(II)-tetrakis(4-N-methylpyridyl)

porphyrin, tetrachloride salt (CuTMPyP4), and Cu(II)-5,10,15,20-tetrakis(4-sulphonatophenyl) porphyrin, tetrasodium salt (CuTSPP4), were purchased from Frontier Scientific (Logan, U.S.A.). Cellulose acetate membrane (cut-off = 12 kDa), branched polyethylenimine (PEI, MW = 25000), polyhexamethylene guanidine hydrochloride (PHMG), analytical grade DMSO, NaI, LiCl, AgNO3, sodium mercaptoethyl sulfonate, mercaptopropionic acid, 2-mercaptoethanol, 2-(dimethylamino)ethanethiol hydrochloride, acetylcholine chloride, sodium citrate and other reagents, if not mentioned otherwise, were purchased from Merck and used without additional purification.

Instrumentation and Software. Absorption and extinction spectra of the samples were measured by PB 2201 spectrometers (SOLAR, Belarus). Scanning electronic microscopy (SEM) images were performed by Zeiss LEO SUPRA 25 (Germany). Transmitting electron microscopy (TEM) images were performed by Zeiss LEO 906E (Germany). SEM and TEM images were treated by ImageJ 1.51k freeware. AFM images were scanned in air using BioScopeResolve (Bruker) atomic force microscope in PeakForceQNM mode with recording the adhesion force maps and topographic images. SERS measurements were carried out by using a scanning probe Raman microscope "NanoFlex" (Solar LS, Belarus). The source of excitation at 488.0 nm was an argon ion laser (Melles Griot, USA). Excitation and registration of Raman scattering was carried out through 100× objective and with CCD camera Newton 970 EMCCD DU970P-BV (Andor Technology Ltd, UK). Additionally, in some cases, the SERS spectra were taken using Raman spectrometer equipped with Spex 270M (Jobin Yvon) spectrograph and liquid-nitrogen cooled CCD detector (Princeton Instruments). Spectra were excited by a 441.6 nm of a He-Cd laser.

Synthesis of silver NPs. 50 ml of deionized water, 42 mg of sodium bicarbonate, 15 mg sodium citrate and 450 mg of water-free glucose were placed into round bottom flask and stirred for complete dissolution. Then, 640 µl of 4 mg/ml silver nitrate solution were added under

vigorous stirring and kept for 4 h in the ultrasonic bath. Resulting silver nanoparticles solution was dialyzed against 2.5 mM sodium citrate and stored at 4°C.

Glass and silicon surface functionalization. Standard optical microscope glass slides of 1.0 mm thickness were used as a base substrate. As a silicon support, 5 mm square-shaped polished chips of Sb-doped electrically conductive silicon was used. The same modification protocol was applied as for the glass, and for the silicon supports.

The substrates were treated by 1:1 (by mass) mixture of concentrated H2SO4 and 30% hydrogen peroxide water solution for 2 h, rinsed with distilled water and dried. Cleaned substrates were treated with (3-chloropropyl)trichlorosilane water-free toluene solution (2% by mass) during 24 h and rinsed several times by dry toluene, then by isopropyl alcohol, and dried at room temperature. The chloropropyl-functionalized substrates were then modified with PEI by soaking in the 10% (by mass) polymer solution in DMSO at 80°C for 5 h in the presence of catalytic quantity of sodium iodide. Finally, substrates were repeatedly rinsed with hot distilled water, and dried.

Silver NPs adsorptive immobilization kinetics study. PEI-modified glass substrate 5×10 mm in size was placed into 3 ml (great excess) of Ag NPs (Ag concentration of 32 μg/ml) water solution with 2.5 mM sodium citrate and kept at room temperature. A 2.5 mm quartz cuvette filled with 2.5 mM sodium citrate water solution was used to get absorption spectra. The glass substrate covered by Ag NPs was placed into the cuvette following washing by citrate buffer. Spectrum was immediately registered, and the substrate was returned into the Ag NPs solution. The absorption spectra were obtained against to the analogous blank glass substrate.

Chemical modification of plasmonic surface. PEI-modified glass substrates were kept in Ag NPs water solution (silver concentration of 32 μg/ml) with 2.5 mM sodium citrate at room temperature for 24 h. Resulting substrates were washed with deionized water and further

chemically treated or dried for use without additional modification. The same protocol was applied for silicon substrate preparation to get NPs SEM images.

Glass-based SERS substrates were treated with 0.1 mg/ml solutions of a series of organic reagents (sodium mercaptoethyl sulfonate, mercaptopropionic acid, 2-mercaptoethanol, 2-(dimethylamino)ethanethiol hydrochloride, acetylcholine chloride, PEI, PHMG). Thiocholine was generated in situ by basic hydrolysis of its acetyl ether in sodium carbonate buffer with pH=10.8. The supports were soaked during 30 min in the corresponding reagent solution, washed by deionized water and dried. Binary charged metal ions treatment was performed in 5 mg/ml copper (Cu) or magnesium (Mg) sulfate solution during 30 min with the following washing and drying. Lithium chloride treatment was performed according to the reported protocol [14].

Preparation of samples for SERS analysis. To assess the SERS substrate activity, water solutions of cationic CuTMPyP4 (1 μM), anionic CuTSPP4 (1 μM), or oligonucleotide (10 μM) was used as a probe analyte. The solution was placed onto substrate by the drop-casting method (ca. 100 μl/cm2), and dried at room temperature. In the case of the metal ion addition, 5 mM of the corresponding sulfate were introduced to the analyte solution.

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