Regular Article

# Plasmon mediated decomposition of brominated nucleobases on silver nanoparticles – A surface enhanced Raman scattering (SERS) study<sup>\*</sup>

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**Abstract.** The localized surface plasmon resonances (LSPRs) of silver nanoparticles (AgNPs) give rise to the generation of so called hot electrons and a high local electric field enhancement, which enable an application of AgNPs in different fields ranging from catalysis to sensing. Hot electrons generated upon the decay of LSPRs are transferred to molecules adsorbed on the surface of the NPs and trigger chemical reactions via dissociative electron attachment (DEA). Herein, we report on the hot electron induced decomposition of the brominated nucleobases – 8-bromoadenine, 8-bromoguanine, 5-bromocytosine and 5-bromouracil on laser illuminated AgNP surfaces. Surface enhanced Raman scattering (SERS) spectra of all canonical nucleobases and their brominated analogues have been recorded at different laser illumination times, and for the very first time we present SERS measurements of 8-bromoguanine and 5-bromocytosine. Reaction products have been identified by their vibrational fingerprint revealing the cleavage of the carbon bromide bond in all cases even under mild illumination conditions. These results indicate that the wellknown reactions from DEA experiments in the gas phase (i) are also taking place on nanoparticle surfaces under ambient conditions, (ii) can be monitored by SERS, and (iii) are also of importance in analytical SERS applications involving electrophilic molecules, as the bands originating from reaction products need to be identified.

# 1 Introduction

Collective oscillations of the conduction band electrons in noble metal nanoparticles (NPs), so called localized surface plasmon resonances (LSPRs), are excited by the alternating electric field of incident visible or near-infrared light. LSPRs enhance the local electric field in the vicinity of the NPs. This effect is exploited in surface enhanced Raman scattering (SERS), where the initial Raman signal of molecules on the NP surface is enhanced by the forth power of the electric field enhancement  $|E/E_0|$  [1]. The combination of the high signal enhancement and the specificity due to the vibrational fingerprint of the molecule enables analytical applications using SERS [2–4]. For analytical applications it is crucial to differentiate between the signals of the analyte molecules and signals of reaction products generated during the measurement process. Such reactions can be initiated by an electron transfer, as

besides the scattering process utilized in SERS, LSPRs can also decay in a non-radiative pathway by forming electron-hole pairs [5–8]. These electrons mainly generated by Landau damping at the surface of the NPs can be further transferred into an unoccupied molecular orbital of adsorbed molecules and trigger reactions therein [9,10]. As the energy of these electrons exceeds for a short timescale the thermal equilibrium of the AgNP lattice, they are typically referred to as hot electrons [5]. Alternatively, also a direct electron transfer from the NPs into the molecular orbitals by a chemical induced damping of the LSPRs is possible [11]. The occupation of a formerly unoccupied molecular orbital results in the formation of a transient negative ion, which can decay into a negative and one or more neutral fragments via dissociative electron attachment (DEA) [12]:

$$AB + e^- \rightarrow AB^{-\#} \rightarrow A^- + B$$
.

DEA to molecules in the gas phase is typically studied using crossed molecular-electron beam setups and mass spectrometry [12] revealing a high energy and bond selectivity of the DEA process [13,14]. The adsorption of molecules on a surface strongly influences the DEA process, e.g. due to trapping of the negative charge in image

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analogues.

potential states [15,16]. Even though the precise energy of the transferred electrons in hot electron induced reactions remains unknown, it is limited by the initial photon energy (< 3 eV) and typically close to the Fermi energy [8]. In consequence, only low-lying resonances are involved in the formation of the transient negative ions. Within this work, we are studying the hot electron induced decomposition of brominated nucleobase analogues on AgNP aggregates. Bromoadenine (BrA) [17] and bromouracil (BrU) [18] in the gas phase show a high reactivity towards low energy electrons (LEEs) with an energy close to 0 eV resulting in the cleavage of the carbon bromide bond leaving the residual molecule intact. For bromocytosine (BrC) and bromoguanine (BrG) equivalent reaction pathways are postulated [19–21]. On the contrary, DEA measurements in the gas-phase on the nucleobases adenine [22], cytosine [23], guanine [24] and thymine [23] do not exhibit resonant bond cleavage upon attachment of LEEs close to 0 eV. In these cases the most abundant fragmentation channel is the abstraction of an hydrogen atom induced by electrons with an energy around 1 eV [22-24]. Recently, the breaking of the C-Br bond in BrU has been observed in the proximity of AuNPs irradiated by intense laser pulses, most likely induced by thermionically emitted LEEs [25]. Moreover, it has been demonstrated for BrA that the electron induced cleavage of the C-Br bond can be observed on AgNPs when illuminated with low intensity continuous wave laser light [26]. Halogenated nucleobases are of special interest due to their strong interaction with LEEs and potential applications as radiosensitizing agents in cancer therapy [27]. In that context is known that the combination of electrophilic radiosensitizers with NPs, as a source of electrons, highly enhances radiation induced DNA damage [28]. Beyond that, metal NPs illuminated by lasers are proposed as a novel therapy in cancer research, the so called photothermal therapy (PTT), where cancer cells are effectively killed due to the elevated temperatures in the vicinity of the NPs [29]. However, in the context of PTT the role of hot electrons has poorly been studied and consequently the application of electrophilic radiosensitizers has not been considered so far. Hence, in the presented work the plasmon mediated reactions of brominated nucleobases have been examined by SERS. The reactions were triggered and tracked by the laser in a confocal Raman microscope setup [30]. Therefore, we have decorated AgNPs with brominated and non-brominated nucleobases and measured their behavior towards hot electrons generated by laser light on the surface of aggregated NPs through SERS in the condensed phase at different illumination times. The vibrational fingerprint of the reaction products has been determined, in order to enable future experiments in more complex environments, which are required to evaluate the potential role of radiosensitizing agents in PTT. Furthermore, a time dependent study for BrA was carried out to identify the reaction products formed during the dissociative hot electron transfer by monitoring the change in intensity of respective vibrational bands of nucleobases and their brominated

# 2 Experimental section

## 2.1 Chemicals

Adenine (A), cytosine (C), guanine (G), uracil (U), bromocytosine (BrC), bromoguanine (BrG), bromouracil (BrU), silver nitrate (AgNO<sub>3</sub>) and trisodium citrate were purchased from Sigma Aldrich (Germany). Bromoadenine (BrA) was purchased from Carbosynth (UK). All chemicals were used in Milli-Q filtered water without further purification.

#### 2.2 AgNP synthesis

AgNPs were synthesized by citrate reduction method using a modified Lee-Meisel protocol [31]. The glassware was cleaned with *aqua regia* prior to use. 90 mg of AgNO<sub>3</sub> was dissolved in 500 mL water and the solution was heated up to a rolling boil under reflux condition in a 1000 mL three neck round bottom flask (T = 125 °C). This was followed by addition of 10 mL of 4% aqueous trisodium citrate to the boiling solution rapidly using a syringe under stirring condition (600 rpm). The boiling was further continued for 1 more hour. Subsequently, the solution was cooled down to room temperature and stored at 4 °C for further experiments.

#### 2.3 UV-Vis spectroscopy

UV-Vis-extinction spectra were recorded with a Nanodrop 2000 from Fisher Scientific.

## 2.4 Atomic force microscopy (AFM)

AFM technique was used to determine the size of the AgNPs. A 2  $\mu$ L droplet of AgNPs, which had been washed with MilliQ water, was drop cast on a Si substrate and allowed to air-dry. Next, the size distribution of AgNPs was determined using the height profiles of the particles obtained from AFM images. The AFM images were recorded using a Flex AFM from Nanosurf equipped with Tap150 Al-G (5 N/m, 150 kHz) cantilever in the soft tapping mode. The data processing was performed using the Gwyddion (v 2.48) software.

#### 2.5 Raman spectroscopy

Sample preparation: the as synthesized AgNPs were purified by centrifugation prior to use. Briefly, 1 mL Ag NP dispersion was taken and centrifuged at 2000 rcf for 10 min at 20 °C and the precipitate was re-dispersed in 1 mL MilliQ water. To prepare samples for Raman measurements typically 20  $\mu$ L of 0.5 mM solution of three nucleobases (A, G and U) and their brominated analogues (BrA, BrG and BrU) were mixed with 80  $\mu$ L of AgNPs



**Fig. 1.** UV-Vis extinction spectrum of the freshly synthesized AgNPs.

dispersion. In case of C and BrC, 10  $\mu$ L of 0.5 mM were mixed with 90  $\mu$ L of AgNPs dispersion. The 0.5 mM G and BrG solutions were made in NaOH, due to their poor water solubility, such that the final concentration of NaOH was 13 mM and 0.8 mM in the respective solution. The resulting mixture was then allowed to incubate for 1 h followed by centrifugation at 1000 rcf for 10 min at 20 °C. The precipitate obtained after centrifugation was re-dispersed in 20  $\mu$ L Milli-Q water and 10  $\mu$ L dispersion was drop cast on a cleaned Si wafer and allowed to air-dry overnight.

Raman measurements: Raman measurements of nucleobase coated AgNPs were carried out using a WITec alpha300 Raman-microscope with a 532 nm excitation laser and  $10 \times$  objective (NA = 0.25). For all measurements, laser power of 50  $\mu$ W was used. The measurements were carried out in triplicate from different sample area. The normal Raman spectra (NRS) of the solid nucleobase samples were recorded using a 785 nm laser with 1 mW power (5 s integration time, 5 accumulations).

*Data processing:* the SERS spectra were analyzed and processed using Origin 9.1 software.

# 3 Results and discussion

Figure 1 shows the extinction spectrum of the freshly synthesized citrate stabilized AgNPs. The extinction maximum located at 411 nm with a full-width at half maximum of 103 nm indicates the formation of AgNPs with an average diameter of 30 nm [32].

The size of the NPs was also determined by AFM measurements from the height profile of dried AgNPs imaged on a Si wafer revealing a diameter,  $d = (34 \pm 9)$  nm. Figure 2 shows an AFM image of the AgNPs and the size distribution of the as synthesized AgNPs in the inset.

In order to monitor the plasmon mediated reactions by SERS, the AgNPs were coated with the nucleobase and their brominated analogues which were then drop cast on a silicon substrate and allowed to air dry. The dried samples formed fractal aggregates of AgNPs, which are known for high optical enhancement [33]. The aggregation process is strongly influenced by the type of the nucleobase [34,35]. During the incubation and washing steps the AgNPs tend



**Fig. 2.** AFM image of AgNPs on Si Wafer. Inset shows size distribution of as-synthesized AgNPs determined from AFM images fitted with a Gaussian function (red).

to aggregate, as on the one hand the adsorbed nucleobases interact with each other e.g. due to  $\pi - \pi^*$  stacking interaction and on the other hand the citrate as surface stabilizer is removed [36.37]. The latter is crucial to minimize the contributions of the citrate molecules to the Raman signal and also to facilitate adsorption of the nucleobases on the NP surface. In consequence, the AgNP solution gets relatively unstable due to the ligand exchange and needs to be rapidly processed. Therefore, after a final centrifugation step, the nucleobase coated AgNPs are immediately dried on a Si substrate. Figure 3 shows the bright-field microscopic images of all the AgNPs coated with nucleobases and their brominated analogues, revealing a general influence of the nature of nucleobases on the formation process of the SERS substrate. Consequently, in order to ensure reproducible Raman measurements, densely covered areas with a similar contrast in the brightfield microscopic images have been chosen for all measurements. It is to be mentioned at this point that a direct quantification and comparison between the observed reactions is challenging. In order to quantify the measurements with the different nucleobases, their Raman cross-section, the local signal enhancement on the nanoscale and the concentration, position and orientation of the adsorbed nucleobases in the reactive regions [38] need to be determined.

Surface enhanced Raman scattering spectra of the AgNP samples coated with halogenated nucleobases were recorded at different illumination times using a 532 nm laser (50  $\mu$ W) focused with a 10× / 0.25 NA objective to a spot with approximately 2.6  $\mu$ m diameter. Mild illumination conditions have been chosen in order to avoid effects due to electric fields, which are even in the hot spots well below 10<sup>7</sup> V/m, and moreover minimize the heating of the substrate. Photolysis of the molecules is unlikely as well, since the molecules do not absorb light in the visible range and two-photon processes are not relevant at the present photon densities [35].



Fig. 3. Brightfield microscopic images of the aggregated AgNPs capped with different nucleobase analogues using a Nikon E-Plan  $50 \times /NA = 0.75$  objective. Inset in the guanine image shows a digital image of guanine capped AgNPs dried on a Si wafer.

## 3.1 Bromoadenine and adenine

The results for BrA reported herein are in accordance with measurements under slightly different experimental conditions published previously [26]. Figure 4 clearly reveals the vibrational band at  $770 \text{ cm}^{-1}$  assigned to the ring breathing mode of BrA at the beginning of the illumination (1 s) of the sample. On the other hand, the SERS spectrum recorded after 100 s of laser illumination, shows a sharp decrease in the intensity of the BrA ring breathing mode, and at the same time peaks associated with the vibrational fingerprint of adenine appear clearly. Table 1 shows all observed bands in the SERS spectrum of BrA on AgNPs which have been compared with the NRS spectrum of solid BrA. The peak assignment has been done with respect to the vibrational frequencies determined by Chen et al. using density functional theory (DFT) calculations [39].

The most prominent band observed after 100 s illumination time was due to the ring breathing mode of adenine at  $739 \text{ cm}^{-1}$ . Further prominent new bands, clearly associated with the formation of A are observed at  $638 \text{ cm}^{-1}$ ,  $965 \text{ cm}^{-1}$  and at  $1341 \text{ cm}^{-1}$ . The assignments for all the observed Raman bands are shown in Table 1. Slight deviations in the wavenumbers are observed for the rising A peaks during decomposition of BrA compared to the SERS signal of only A recorded on AgNPs as reported in Table 1. These deviations may be explained by the close overlap of A peaks with the BrA peaks in the SERS spectra or due to the possible different orientation of the molecules on the NP surface. Moreover, also modifications of the A molecule such as A radicals or charged species are possible. During the reaction process, the bands due to BrA underwent a decrease in intensity, particularly pronounced at the ring breathing mode at 770  $\rm cm^{-1}$ , while the vibrational bands due to A increase. Even at a comparatively low laser power and within 1 s of laser illumination, the SERS spectra showed Raman peaks, which originated from the vibrational modes of A on the AgNPs surface.



Fig. 4. SERS spectra of BrA adsorbed on AgNPs after 1 s (light red) and 100 s (black) of laser illumination in a confocal Raman microscope and SERS spectrum of A on AgNPs (dark red) is shown as a control. Laser wavelength: 532 nm, laser power: 0.05 mW, data acquisition time: 0.4 s, number accumulations: 3. The wavenumbers of the marked Raman bands are determined from the BrA spectrum after 100 s of laser illumination. Molecular structure of A and BrA are presented on the right side.

The A is most likely formed by a dissociative hot electron transfer from the laser illuminated particles:

$$AgNPs + hv \to e^- + h^+.$$

The electron is captured by BrA and forms a metastable anion, which decays mainly by the cleavage of the C–Br bond if the incident electron energy is low [17]:

$$BrA + e^- \rightarrow BrA^{\#-} \rightarrow Br^- + A$$

The reaction was tracked by following the decrease in the intensity of the ring breathing mode of BrA at  $770 \text{ cm}^{-1}$ 

**Table 1.** Raman shifts of NRS and SERS bands of A and BrA observed in the present experiment given in cm<sup>-1</sup> and assignment of the bands according to references [39] and [40].  $\omega$ : wagging;  $\delta$ : in-plane deformation;  $\Delta$ : in-plane ring deformation of skeletal atoms;  $\gamma$ : out-of-plane deformation;  $\Gamma$ : out-of-plane ring deformation of skeletal atoms;  $\alpha$ : scissoring;  $\tau$ : torsion; t: twisting;  $\nu$ : stretching; r: rocking; Pyr: pyrimidine; Im: imidazol. Atoms are labeled according to Figure 4.

NRS A	SERS A	Assignment [40]	NRS BrA	SERS BrA	Assignment [39]
193		$\gamma( ext{C6-N10})$	153		$\delta(\text{C8-Br}),\delta(\text{C6-N10}),\delta(\text{Pyr})$
246		"Butterfly" mode	198		
333		$\Delta$ (C6–C5–N7) – $\Delta$ (N1–C6–N10) + $\Delta$ (N3– C4–N9)	248		$\nu$ (C8–Br), $\delta$ (C6–N10), $\delta$ (Im), $\delta$ (C8–Br)
539		$\Delta(N1-C6-C5) + \Delta(C2-N3-C4)$	298		$\tau$ (C4–C5) $\gamma$ (C8–Br) $\tau$ (Pyr ring)
564	568	$\Gamma(N1-C2-N3) + \alpha(C2-H) - \alpha(C8-H)$	379		$\Lambda(C6-N10) = \mu(C8-Br) = \delta(C8-Br)$
004	508	1(11-02-113) + 9(02-11) - 9(03-11)	512		$\Delta(CO-NIO), \nu(CO-DI), \nu(CO-DI),$
222	0.14				$\alpha(\min \operatorname{Fing})$
626	641	$\Delta(C4-C5-N7) - \Delta(C4-N9-C8) + \Delta(N1-C6-N10)$	547		$\delta(\text{Pyr Ring}), \delta(\text{C8-Br}), \delta(\text{C6-N10})$
727	740	Pyr + Im ring breathing	596	572	$\tau$ (Pyr Ring), $\tau$ (Im Ring), $\gamma$ (C6–
		()			N10)
801		$\omega({ m NH2})$	628		$\tau$ (Pyr Ring), $\tau$ (Im Ring), $\gamma$ (C6–
					N10)
902	933	$\Delta$ (N1-C2-N3) + $\Delta$ (C8-N9-C5)	757	770	Pyr + Im ring breathing
945	957	$\Delta$ (N7–C8–N9); $\gamma$ (N9–H)	894		$\delta$ (Pvr Ring), $\nu$ (C5–N7)
1028	1035	$t(NH2): \nu(C6-N1)$	913		$\delta(\text{Im}), \delta(\text{Pyr}), \nu(\text{C8-N9})$
1120	1137	$\delta(C_{8}-H)$ ; $\nu(C_{3}-N_{3}) + \nu(C_{4}-N_{0})$	084	030	$r(NH2) \mu(C6-N1)$
1125	1157	$\delta = 0(05-11), \nu(05-105) + \nu(04-105)$	304	333	$\nu(C2, N1)$
1100		$0 - \nu(C3 - C0) - \nu(C3 - N7)$	1020	1091	$\nu(C2-N1)$
1100		$\rho(C8-H) = \rho(N9-H); \nu(C8-N9) + (C5-N7) = \nu(C4-N0)$	1039	1031	$\nu(C8-N9), o(N9-H), \nu(C4-N9)$
		$N9) - \nu(C4-N3) - \nu(C2-N3)$			
1252	1280	$\delta(C8-H) + \delta(N9-H); \nu(C2-N3) + \nu(C8-N7) +$	1148		$\delta(N9-H), \nu(C4-N3), \delta(Im ring),$
		u(C2-N1) - u(C5-N7) - u(C4-N9)			$\nu$ (C4–N9)
1312		$\delta(\text{C2-H}); \nu(\text{C2-N3}) + \nu(\text{C5-N7}) - \nu(\text{C8-N7}) -$	1176		
		$\nu$ (C4–N9)			
1336	1346	$\delta$ (C2-H); $\nu$ (C2-N3) + $\nu$ (C5-N7) - $\nu$ (C8-N7) -	1210		
		$\nu$ (C4–N9)			
1375		$\delta$ (N9–H): $\delta$ (C8–H): $\nu$ (C8–N9) + $\nu$ (C4–C5) + $\nu$ (C2–	1256	1257	$\nu$ (C5–N7), r(NH2), $\nu$ (C2–N3),
1010		N(2) + u(C6-N1) - u(C5-C6) - u(C2-N1) - u(C4-C6-N1) - u(	1200	1201	u(C2-N1)
		$N_{3} = \nu(C_{3} - N_{1}) - \nu(C_{3} - C_{0}) - \nu(C_{2} - N_{1}) - \nu(C_{4} - N_{2})$			$\nu(02-101)$
1400		N(3) = V(C(3-N(1)))	1004		(C2 N2) = (CT N7)
1422		$\nu(0) = \mu(0) = \nu(0) = $	1294		$\nu(C2-N3), \nu(C5-N7)$
		N3) + $\nu$ (C6–N1) – $\nu$ (C5–C6) – $\nu$ (C2–N1) – $\nu$ (C4–			
		$N3) - \nu(C8-N7)$			
1466	1464	$\delta$ (N9–H) – $\delta$ (C8–H) + $\delta$ (C2–H); $\nu$ (C8–N9) + $\nu$ (C2–	1330	1332	$\delta$ (C2–H), $\nu$ (C6–N10), $\nu$ (C8–N9),
		N1) + $\nu$ (C6–N1) - $\nu$ (C2–N3) - $\nu$ (C8–N7) - $\nu$ (C6–			$\nu$ (C2–N1)
		N10)			
1486		$\alpha$ (NH2); $\delta$ (N9–H) $\delta$ (C8–H) + $\delta$ (C2–H); $\nu$ (C4–C5)	1351	1388	$\delta$ (N9–H), $\nu$ (C4–N9), $\nu$ (C8–N9)
		$+ \nu(C2-N1) + \nu(C6-N1) + \nu(C8-N9) \nu(C2-N3)$			
		$\nu(C4-N9)$ $\nu(C8-N9)$			
1601		$\alpha(\text{NH2}): \delta(\text{N9-H}): u(\text{C4-C5}) \perp u(\text{C2-N1}) u(\text{C5-C6})$	1445	1/135	u(C8-N7)
1001		u(06  N1) u(04  N2) u(09  N2)	1440	1400	
1015	1094	V(00-N1) V(04-N3) V(02-N3)	1454		
1015	1634	$\alpha(\text{NH2}); o(\text{N9-H}); \nu(\text{C4-C5}) + \nu(\text{C2-N1}) - \nu(\text{C5-C6})$	1454		
		$V_{0} = \nu(V_{0} = N_{1}) - \nu(V_{4} = N_{3}) - \nu(V_{2} = N_{3})$			
			1494	1497	$\delta(C2-H), \nu(C6-N1), \nu(C6-N10),$
					$\delta(NH2)$
			1524	1553	$\Delta$ (NH2), $\nu$ (C4–C5), $\nu$ (C5–C6)



Fig. 5. Intensities of the ring breathing modes of BrA on AgNPs at 770 cm<sup>-1</sup> (black) and A at 739 cm<sup>-1</sup> formed under laser illumination (light red) of BrA plotted as a function of the illumination time. The intensity of the ring breathing mode of A on AgNPs as a control sample (dark red).

and the concomitant rise of the A peak at 739 cm<sup>-1</sup>, respectively. After approximately 20s of illumination, both A and BrA contributed equally to the measured signal in the SERS spectra (see Fig. 5).

Additionally, as a control, A has been adsorbed on AgNPs and illuminated with laser light as shown in Figure 5. The Raman intensity at 740 cm<sup>-1</sup> due to ring breathing mode of the A signal was monitored. The rise in the signal intensity observed within in the first few seconds of laser illumination might be explained by a charge transfer from the AgNP to the adsorbed A leading to the chemical enhancement of the Raman signal [41]. After the initial signal rise, the signal intensity due to ring breathing mode of A remained constant, indicating that neither the molecules are decomposed nor the plasmonic substrate is modified.

#### 3.2 Bromocytosine and cytosine

Raman spectra of BrC and C on AgNPs have been recorded under the same measurement conditions as

**Table 2.** Raman shifts of NRS and SERS bands of C and BrC observed in the present experiment given in cm<sup>-1</sup> and assignment of the bands according to references [44] and [43].  $\omega$ : wagging;  $\delta$ : in-plane deformation;  $\Delta$ : in-plane ring deformation of skeletal atoms;  $\gamma$ : out-of-plane deformation;  $\Gamma$ : out-of-plane ring deformation of skeletal atoms;  $\alpha$ : scissoring;  $\tau$ : torsion; t: twisting;  $\nu$ : stretching; r: rocking; ring: pyrimidine ring. Atoms are labeled according to Figure 6.

NRS C	SERS C	Assignment [44]	NRS BrC	SERS BrC	Assignment [43]
162			229		
237			294		
407			312	307	$\delta$ (C–Br), ( $\Delta$ (ring), $\delta$ (NH2)
448	440		408		puckering N3 + $\gamma$ (NH2)
540	494		428	427	$\gamma$ (N1-CH=C), ( $\Gamma$ (ring), $\tau$ (NH2)
552	565	$\Delta(\text{ring})$	546	477	$\Delta$ (ring) mainly in N1, N3 + $\delta$ (NH2)
603	605	$\gamma(C2=O)$	588	580	$\Delta$ (ring), $\delta$ (C=O), $\delta$ (NH2)
702		$\Gamma(ring)$		624	$\gamma$ (N1–H), $\Gamma$ (ring), $\tau$ (NH2)
794	801	ring breath	662	681	$\gamma$ (N–CO–N) mainly in C2, $\Gamma$ (ring)
896				738	
975			788	802	$\nu$ (ring), $\delta$ (NH2)
993	1009		867		
1014	1040		879		
1112	1135		949	953	$\gamma({ m C6-H})$
1153	1199	$\Delta$ (ring), $\nu$ (C–N)	979	1009	$\nu$ (ring)
1252	1253	$\Delta$ (ring), $\nu$ (C–N)	1064	1067	
1279	1310	$\Delta$ (ring), $\nu$ (C–N)		1116	
1365	1368	$\delta$ (N1–H, C5–H, C6–H)	1236	1177	$\delta$ (H–N1–C6–H), $\nu$ (ring), $\delta$ (NH2)
1464	1429	$\delta$ (N1–H, C5–H, C6–H)	1283	1244	$\nu$ (C2–N3), $\nu$ (CN1), $\Delta$ (ring), $\delta$ (NH2)
1537	1489	$ u({ m C4-N}) $	1308	1308	$\delta$ (C–H), $\nu$ (CCC), $\delta$ (N1–H)
1656	1591	$\delta(\text{NH2})$		1340	
1694	1637	$\nu(C2=O)$	1444	1467	$\delta(\text{N1-H}), \nu(\text{N-C4}), \Delta(\text{ring}), \delta(\text{NH2})$
			1537		$\nu$ (N3–C4), $\nu$ (N1–C–C), $\tau$ (NH2)
			1622		
			1647		$\nu$ (C=C), $\nu$ (C–N), $\tau$ (NH2)



Fig. 6. SERS spectra of BrC adsorbed on AgNPs after 2 s (light green) and 600s (black) of laser illumination in a confocal Raman microscope. A SERS spectrum of C on AgNPs (dark green) is shown as a control. Laser wavelength: 532 nm, laser power: 0.05 mW, data acquisition time: 2 s, number accumulations: 3. The wavenumbers of the marked Raman bands are determined from the BrC spectrum after 600 s of laser illumination. Molecular structure of C and BrC are presented on the right side.

reported for BrA and A in Section 3.1. All observed bands are listed in Table 2 and have been assigned to the vibrational modes of the molecules reported previously by DFT calculations [42,43]. It is to be mentioned that the ring breathing modes of BrC and C appear at 802 cm<sup>-1</sup> and



Fig. 7. SERS spectra of BrG adsorbed on AgNPs after 1 s (yellow) and 110 s (black) of laser illumination in a confocal Raman microscope. SERS spectrum of G on AgNPs (brown) is shown as a control. Laser wavelength: 532 nm, laser power: 0.05 mW, data acquisition time: 0.4 s, number accumulations: 3. The wavenumbers of the marked Raman bands are determined from the BrG spectrum after 110 s of laser illumination. Molecular structure of G and BrG are presented on the right side.

 $801 \text{ cm}^{-1}$ , respectively. As a result, the intensity of the Raman signal appears to be constant during the illumination, even if a transformation from BrC to C occurs considering comparable Raman cross sections of both molecules. In consequence, the plasmon mediated decomposition of BrC is more difficult to track compared to BrA, since there is no distinct intense band of C, that does not overlap with

**Table 3.** Raman shifts of NRS and SERS bands of G and BrG observed in the present experiment given in cm<sup>-1</sup> and assignment of the bands according to reference [45]  $\omega$ : wagging;  $\delta$ : in-plane deformation;  $\Delta$ : in-plane ring deformation of skeletal atoms;  $\gamma$ : out-of-plane deformation;  $\Gamma$ : out-of-plane ring deformation of skeletal atoms;  $\alpha$ : scissoring;  $\tau$ : torsion; t: twisting;  $\nu$ : stretching; r: rocking; Pyr: pyrimidine; Im: imidazole. Atoms are labeled according to Figure 7.

NRS G	SERS G	Assignment [45]	NRS BrG	SERS BrG
173			151	
208			180	
344			292	291
381	390	$\Delta$ (C2–N10)	380	399
404			414	456
499	489	$\Delta(Pyr)$	530	492
552	531	$\Delta(Pyr)$	564	558
564			580	606
609	600	$\Delta(Pyr), \tau(NH2)$	675	695
653	670	Breath Pyr	690	748
697	709	$\Gamma(Pyr), \gamma(C6=O)$	775	770
714	763		867	838
780	794		976	951
850	834	$\omega(\text{NH2}),  \Delta(\text{Pyr}),  \Delta(\text{Im})$	1053	1015
887			1171	1178
943	963	$\Delta({ m Im})$	1189	
	1010		1213	
1054	1049	$\nu$ (N1–C2, C2–N10)	1233	1276
1162		r(NH2)	1346	1332
1188	1199	$r(NH2), \delta(N1-H, C8-H)$	1394	1403
1233	1226	$ u({ m C8-N9}) $	1435	
1266	1245	$\delta$ (C8–H, N1–H), $\nu$ (N7–C8), r(NH2)	1454	
1364	1293	$\delta({ m N1-H, N10-H})$	1525	1497
1391	1346	$\delta$ (C8–H, $\nu$ (C4–N9, C5–C6, N7–C8)	1546	1558
1416	1400	$\delta$ (N1–H, C8–H), $\nu$ (N3–C4, C4–C5, N1–C2, N7–C8)	1571	
1470			1599	
1553	1553	$\alpha$ (NH2), $\nu$ (C2–N3, C2–N10), $\delta$ (N1–H)	1671	
1602	1627		1693	
1683	1654	$\nu$ (C6=O, C5,C6, C2-N3), $\delta$ (N1-H)		

Raman bands of BrC (see Fig. 6). However, in the irradiated BrC sample new bands at 608 cm<sup>-1</sup>, 1311 cm<sup>-1</sup>, 1499 cm<sup>-1</sup> and 1585 cm<sup>-1</sup> are arising, which clearly indicates the formation of C (see Fig. 6). Compared to the measurements of BrA reported above, only minor changes in the SERS signal of the laser illuminated BrC sample was observed even after six times longer illumination time. Furthermore, the intensities of the new bands assigned to the formation of C are fluctuating, which may also indicate consecutive reactions of the C radical. Due to the strong overlap of the C band with the vibrational bands of BrC the traceability of the reaction is limited.

#### 3.3 Bromoguanine and guanine

Next, the decomposition behavior of G and BrG on aggregated AgNPs have been studied. Figure 7 shows the significant change in the SERS spectrum of BrG during ongoing illumination of BrG on AgNPs. This was reflected by decrease in the Raman signal intensity due to ring breathing mode of BrG at 695 cm<sup>-1</sup> and a concomitant rise of a new band at 670 cm<sup>-1</sup>, which can be assigned to the ring breathing mode of G. In addition, the rising bands at 532 cm<sup>-1</sup> and 1346 cm<sup>-1</sup> also indicate the formation of G (see Fig. 7). The observed Raman bands of the G and BrG samples from our experimental results have been summarized in Table 3 and the band assignment for G has been performed by comparing it with val-



Fig. 8. SERS spectra of BrU adsorbed on AgNPs after 1 s (light blue) and 100 s (black) of laser illumination in a confocal Raman microscope. SERS spectrum of U on AgNPs (dark blue) is shown as a control. Laser wavelength: 532 nm, laser power: 0.05 mW, data acquisition time: 0.4 s, number accumulations: 3. The wavenumbers of the marked Raman bands are determined from the BrU spectrum after 100 s of laser illumination. Molecular structure of U and BrU are presented on the right side.

ues reported by [46] in DFT calculations. It is to be noted that no experimental or theoretical Raman data for BrG has been published so far.

**Table 4.** Raman shifts of NRS and SERS bands of U and BrU observed in the present experiment given in cm<sup>-1</sup> and assignment of the bands according to references [46] and [47].  $\omega$ : wagging;  $\delta$ : in-plane deformation;  $\Delta$ : in-plane ring deformation of skeletal atoms;  $\gamma$ : out-of-plane deformation;  $\Gamma$ : out-of-plane ring deformation of skeletal atoms;  $\alpha$ : scissoring;  $\tau$ : torsion; t: twisting;  $\nu$ : stretching; r: rocking; ring: pyrimidine ring. Atoms are labeled according to Figure 8.

NRS U	SERS U	Assignment [46]	NRS BrU	SERS BrU	Assignment [47]
401	399	$\Delta$ ring	144		
433	455	$\delta(C2=O, C4=O)$	155		
538	566	$\delta(\mathrm{ring})$	207		
560	598	$\delta(\mathrm{ring})$	305		
581	608	$\delta(\mathrm{ring})$	428	433	$\delta(CO) + \delta(ring)$
	654	$\delta(\mathrm{ring})$	552	583	$\delta(\text{ring}) + \delta(\text{CO})$
736	708		614	624	
793	806	Ring breath	638	653	$\delta(\text{ring}) + v(\text{C-Br})$
830				692	
868			751	767	
990			789	806	ring breathing
1010	1012	$\delta(\mathrm{ring})$	848		
	1051	$\delta(\mathrm{ring})$	949	958	
1104	1106	$\delta(\text{C5-H, C6-H})$ + $\nu(\text{C5=C6, C6-N1})$	1017	1010	$\delta(\text{N3-H, C6-H})$ + $\nu(\text{C-N})$
1167			1175	1077	$ u(\operatorname{ring}) $
1239	1221	$\delta$ (N1–H, C6–H, C5–H), $\nu$ (C6–N1)	1184	1202	$\delta$ (N1–H, C6–H) + $\nu$ (ring)
	1284	$\nu$ (N3–C4(–C4–C5–C6–N1), $\delta$ (N1–H, C5–H, C6–H)	1232		
1398	1394	$\Delta$ (C6–H, N1–H, C5–H)	1247	1288	$\delta$ (N1–H, C6–H) + $\nu$ (ring)
1422			1345	1329	$\delta$ (C6–H) + $\nu$ (ring)
1461			1410	1394	$\nu(\text{ring}) + \delta(\text{N1-H})$
1508	1536		1454		
1615	1591	$\nu$ (C4=O, C5=C6, C2=O), $\Delta$ (N1–H, C6–H)	1499	1536	$\nu(C5=C6)$
1648	1633	$\nu$ (C2=O) + $\nu$ (C4=O), $\delta$ (N3–H)	1622	1626	$\nu(C4=O)$
1717			1679		

## 3.4 Bromouracil and uracil

Figure 8 shows the changes recorded in the Raman spectrum during the irradiation of BrU coated AgNPs. The bands at 559 cm<sup>-1</sup>, 1278 cm<sup>-1</sup> and 1590 cm<sup>-1</sup> indicate the formation of U (assignment of all SERS bands of U and BrU are given in Tab. 4). Like for all the nucleobase analogues studied above, we expect to observe a hot electron induced cleavage of the C–Br bond in BrU. However, as already observed for the dissociation of BrC in Section 3.2 the new arising bands have a weak intensity and are often overlapped with the bands of BrU. The ring breathing mode was observed at 806 cm<sup>-1</sup> for both of U and BrU, which makes the simple tracking of the reaction following this Raman mode cumbersome.

# 4 Conclusion

SERS spectra of the four nucleobases and their brominated analogues on AgNPs fractal aggregates have been recorded. The respective assignment of the Raman bands has been done using data from DFT calculations published previously [39,40,42–47]. For all four brominated nucleobases the cleavage of the C–Br bond has been observed leaving the residual molecule intact under low intensity laser illumination in a Raman microscope. The reaction products have been identified by their vibrational fingerprint in SERS spectrum and could be assigned to the non-brominated nucleobases. The decomposition is most

likely to proceed via a dissociative hot electron transfer from the AgNPs to the brominated analogues of nucleobases, although thermal dissociation might also partly contribute to the formation of the reaction products. Additional decomposition pathways have not been detected, however, it needs to be mentioned that the identification of minor changes like hydrogen abstraction during the reaction progress via SERS is challenging. The above mentioned plasmon mediated reactions are of importance in the analysis of SERS spectra of electrophilic molecules in general and especially for analytical SERS applications of DNA, as the spectra of the initially present species need to be distinguished from the spectra of photoproducts generated during the measurement. Importantly, for BrA and BrG, a rapid dissociation to A and G respectively has been observed at low laser powers in our study. The reaction of these purine derivatives can be easily tracked by the ring breathing mode of the molecules. Due to the high signal intensities of these modes and their location in a wavenumber regime without much overlap with other bands, this consequently allows to study the reactions of BrG and BrA in more complex systems, e.g. when these molecules are incorporated in a DNA strand. Such more sophisticated experiments will help to evaluate potential applications of electrophilic radiosensitizers in cancer PTT.

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# Author contribution statement

I.B. and R.S. designed the research and conceived the experiments. All measurements and data analysis have been performed by A.D. and R.S. In the preparation of the manuscript all authors were involved. Furthermore, all authors have read and approved the final manuscript.

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