Chapter 6

Excitation off optical resonance: DNA Bases

In order to demonstrate the applicability of TERS for the sensitive detection and investigation of the adsorption behaviour of nonresonant species, the four deoxyribonucleic acid (DNA) bases adenine (A), cytosine (C), guanine (G) and thymine (T) adsorbed at Au(111) have been studied. These nonresonant molecules are of high biochemical relevance for a better understanding of DNA strand interaction and functioning, being the essential components that hold the intertwined DNA strands together. The characteristic Raman fingerprints allow their straightforward identification and differentiation down to a concentration of 0.8 nmol/cm^2. The TER spectra are compared to NR and SER spectra from the literature and the Raman bands assigned accordingly. A closer look reveals that the spectra differ significantly from NR spectra, but also with respect to SER spectra. Vast differences in the signal-to-noise ratio between the respective nucleobase samples were observed due to the different adsorption behaviour of the bases on Au.

As a resonance effect of the molecule no longer adds to the overall enhancement, the charge transfer or chemical enhancement effect becomes more important in addition to the electromagnetic enhancement. Strongly adsorbed nucleobases are expected to give much stronger Raman scattering than weakly adsorbed bases. Because of ill-defined experimental conditions, we refrain from detailed analysis regarding molecular

orientation or interaction with the Au substrate.

6.1 Experimental part

The DNA bases adenine, cytosine, guanine and thymine are purchased from Sigma-Aldrich at analytical grade (purity > 99%). They are used without further purification to prepare 10^{-3} M ethanolic adsorption solutions. Because of the very low solubility of guanine, the concentration of the adsorption solution is lower in this case. Sample solution is taken from the clear part of the saturated solution. Adsorption is performed as described in Chapter 3.

All presented spectra are normalized to full laser power and 1 s integration time. The spectral intensities are scaled to the most intense Raman band. In order to facilitate band assignment, the spectra are presented after background subtraction.

6.2 Introduction to the DNA bases

The chemical structures of the four DNA bases are shown in Fig. 6.1. Adenine and guanine are two-membered purine systems, whereas cytosine and thymine are one-membered pyrimidine systems. All ring systems fulfill the Hückel rule with a 10π- or 6π-electron structure, respectively, they are planar, and all ring atoms are sp^2-hybridized. Therefore, the DNA bases are classified as aromatic systems, which preferably undergo substitution reactions at the ring that do not destruct the aromatic π-electron system. Interaction with the Au surface can take place either weakly via the π-system or more strongly via the electron lonepairs at the hetero (N or O) atoms.

On opposite complementary DNA strands, A and T (G and C) are connected with hydrogen (bridge) bonds to form the base pairs that hold the intertwined DNA strands together.

In a study on the thermal desorption behaviour of the DNA bases, their binding properties on Au were examined.[173] It was found that, in general, the pyrimidines (C, T) desorb at lower temperatures than the purines (A, G) in the order of T << C < A < G. Numerous studies on the adsorption geometry of the nucleobases at metal

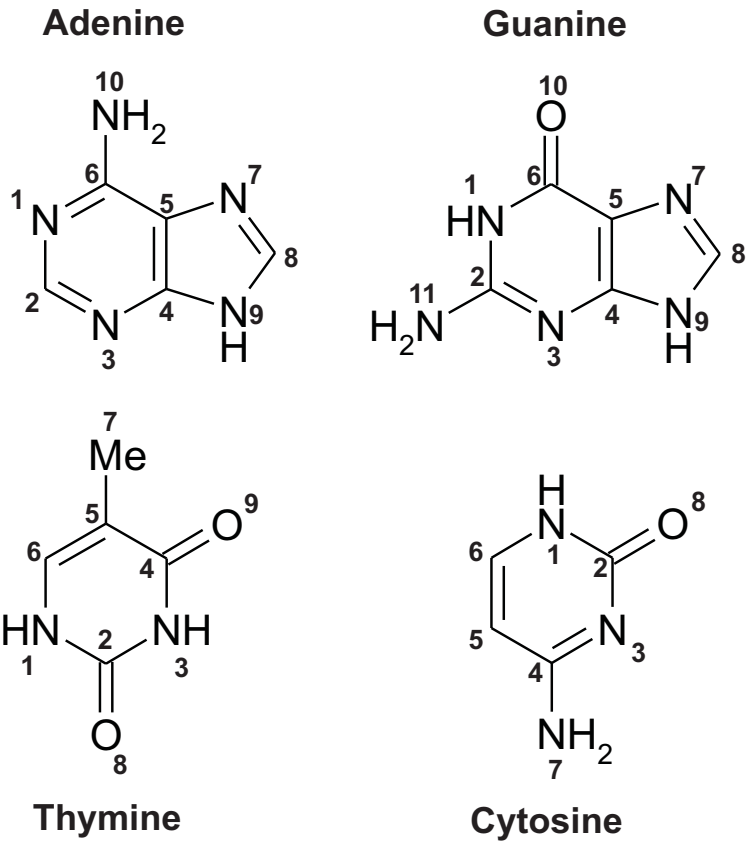

Figure 6.1: The four DNA bases adenine (A), cytosine (C), guanine (G), and thymine (T) exhibit typical functional moieties, like C=N and C=C double bonds, carbonyl or amino groups.

electrodes have been published, but their respective orientations at the surfaces are still under discussion. Regarding our TER studies, the experimental conditions are not yet defined well enough to attempt such an analysis. Therefore, we will refrain from jumping to early conclusions about the molecular orientation at the surface from our data. Instead, we want to emphasize on the highly sensitive identification possibilities of small, nonresonant molecules with TERS. We show that the detection and analysis of comparably small sample amounts (lower than 1 nmol/cm^2), often the limiting factor in biochemical studies, is straightforward: With about 6000 nonresonant molecules present in the enhanced-field region, a fingerprint spectrum with high signal-to-noise ratio can be obtained (see for example Fig. 6.2).

6.2.1 Adenine

Fig. 6.2 shows the TER spectrum of adenine adsorbed at Au(111). Analysis and band assignment are carried out according to Table 6.1, where NR and SER (Ag) data from Giese and McNaughton (Ref. [174]) are listed together with our TER data.

In comparison to the NR spectrum of polycrystalline adenine,[174] a new band has appeared at 227 cm^{-1}. Such bands appearing at low wavenumbers in SER and TER spectra, but not in NR spectra, are generally assigned to the metal-adsorbate bond vibration. A similar peak is found at 233 cm^{-1} in the SER spectrum by Giese and McNaughton for adenine/Ag that is employed here for comparison and band assignment. The similar, but not identical Raman shift indicates a difference in the interaction of adenine with Au and Ag substrates; this difference seems to be quite small in the case of adenine, as band positions and relative intensities are very similar in the TERS and SERS cases.

The TER spectrum is characterized mostly by in-plane vibrational modes, but also some weak out-of-plane modes are identified. The peaks at 339 cm^{-1}, 541 cm^{-1} and 782 cm^{-1} are assigned to out-of-plane ring deformation (torsion, butterfly) and wagging motions (NH_2, C8-H). These modes have a very low polarizability. In order to observe them, the molecule must not be oriented perfectly vertical with respect to the substrate, but must rather adapt a slightly inclined orientation. Only in this way, the strong EM field component in z direction perpendicular to the surface can induce

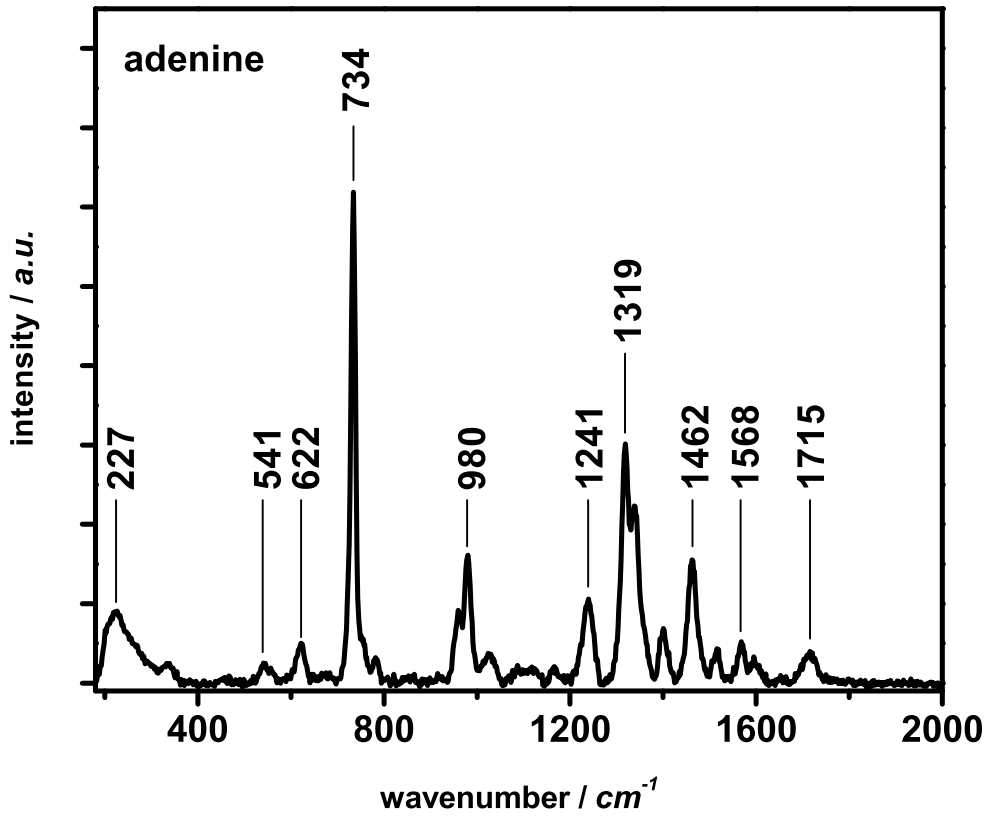

Figure 6.2: TER spectrum (background-corrected) of an adenine (sub)monolayer adsorbed at Au(111). The band at 734 cm^{-1} is the characteristic fingerprint band assigned to the ring-breathing mode of adenine. The excellent signal-to-noise ratio allows the detection of adenine down to lowest adsorbate concentrations of around 65 pmol/cm^2.

a corresponding Raman process. As a result, the out-of-plane modes appear much weaker when the molecule is adsorbed inclined at a surface (TERS and SERS) than in a powder sample, where an average orientation over all possible ones is recorded (NRS).

The most prominent band in the TER spectrum is the one at 734 cm^{-1}. It is assigned to the very characteristic in-plane adenine ring breathing vibration. Its polarizability tensors in x-, y-directions (in the molecular plane) are quite large, resulting in an intense adenine fingerprint band that can easily be detected even in nucleoside or DNA spectra. The comparably large z-component of this mode has to be noted and explains why the corresponding Raman peak is always found in the vibrational spectra, allowing the detection of even slightest traces of adenine.[175] The ring breathing mode is reliably found at 723 cm^{-1} in NR spectra,[140, 174, 176–179] and at around 733 cm^{-1} in SER and TER spectra.[140, 174, 176, 180, 181] The interaction of adenine and a metal surface causes the band to shift upwards, and, in general, slightly higher wavenumbers have been reported for a Au substrate (736 cm^{-1},[181]) than for a Ag substrate (731 cm^{-1},[174]) in SERS, which indicates that the interaction between adenine and Au is a little stronger than between adenine and Ag.

Hayazawa *et al.* published an AFM-TER study on adenine/Ag, where the ring breathing mode is located at 737 cm^{-1}.[179] They ascribe the upshift to the pressure exerted on the adenine molecule by the AFM tip. This is, however, a rather unsatisfying explanation, as also the kind of metal and substrate (and tip) geometries as well as the surface coverage play a role, and these influences were not investigated in detail. Koglin *et al.*, for example, reported on a Raman ring breathing band as high as 740 cm^{-1} for adenine adsorbed at electrochemically roughened Ag.[182]

A few intense bands appear in the spectral region between 1200-1500 cm^{-1} that are assigned to diverse in-plane C=C and C=N double bond stretching modes or N-H and C-H bending modes. The bands appear extremely close in wavenumber with respect to the SER spectrum and are again shifted in comparison to the NR modes due to molecule-surface interactions.

In the uppermost region, between 1515-1715 cm^{-1}, only (very) weak bands appear that are assigned to the NH_2 scissor mode. They show hardly any shift in comparison to the SER and NR spectra, which allows the assumption that the external amino

group movement is not affected much by the adsorption of the molecule and does not itself interact with the metal.

Interestingly, we find one very intense Raman band at 980 cm^{-1}, which is difficult to assign. Despite the band being extremely prominent in the TER spectrum, no corresponding peak is present in the NR spectrum. Giese and McNaughton mention that they sometimes record a Raman peak at 999 cm^{-1} in their SER spectra, but not regularly, which they assign to the wagging of C2-H. If the wagging occurs in the field plane and the C8-H group is located in close proximity to the Au surface, we expect a strongly enhanced Raman band in the spectrum, shifted as before because of the different metal. This may explain that we find the intense 980 cm^{-1} band in the TER spectrum.

In general, obtaining a TER spectrum from an adenine sample was straightforward. In contrast to the other bases, the recorded spectra are extremely intense and show an excellent signal-to-noise ratio. This is likely due to the fact that adenine adsorbs much more strongly at Au than the other nucleobases.[173] First experiments with lower adsorbate concentration testing the sensitivity of TERS for the detection of adenine at Au(111) revealed that only 500 molecules present in the enhanced field region are sufficient to serve as a satisfying TERS sample.

Table 6.1: Assignment of the TER vibrational modes of adenine (ML) adsorbed at Au(111) at pH 7 according to NR and SER spectra by Giese and McNaughton.[174]

mode	NRS	SERS (Ag)	TERS (Au)	plane	assignment
1		br 233 m	br 227 m	-	metal-adenine
2	330 m	325 w	339 vw	in	bend C6-NH_2
				out	butterfly
				out	wag NH_2, def R
				out	tors molecule, wag C6-NH_2
3	536 m	536 vw	541 vw	in/out	def R6 (sqz group N1-C6-C5, C2-N3-C4)
				in/out	wag N9-H, def R6, R5 (sqz group N3-C4-N9)
4	623 m	621 w	622 w	in	def R6 (sqz group C4-C5-C6, N1-C6-N10), R5 (sqz group C5-N7-C8)
5	723 vs	731 vs	734 vs	in	ring breath whole molecule
6	797 vw	788 vw	782 vw	out	def R6 (wag C4-C5-C6), wag C8-H
7	942 m	961 w	960 m	in	def R5 /sqz N7-C8-N9)
8		999[a]	980 s	out	wag C2-H
9	1025 w	1029 w	1026 w	in	rock NH_2
10	1234 s	1245 vw	1241 m	in	rock NH_2, str C5-N-7, N1-C2, C2-N3
11	1308 w		1319 s	in	str C2-N3, N1-C2, C5-C6, C5-N7
12	1333 s	1336 m	1339 s	in	str C5-N7, N1-C2, bend C2-H, C8-H
13	1372 w	1372 w		in	bend C2-H, N9-H, str C8-N9, C4-N9
14	1419 vw	1399 vw	1400 m	in	str C4-N9, C4-C5, C6-N10, N7-C8, bend C2-H
15	1463 vw		1462 s	in	str C2-N3, N1-C6, bend C2-H, sciss NH_2
16		1516 vw	1515 w	in	sciss NH_2
17		1545 vw	1568 w	in	str N3-C4, N1-C6, C5-N7, N7-C8, bend N9-H
18	1597 vw		1595 vw	in	sciss NH_2
19	1674 vw		1715 w	in	sciss NH_2, str C6-N10, C5-C6

[a] Observed irregularly.

6.2.2 Cytosine

Fig. 6.3 shows the TER spectrum of cytosine adsorbed at Au(111). Analysis and band assignment are carried out according to Table 6.2, where NR and SER (Ag) data from Otto *et al.* (Ref. [140]) are listed together with our TER data. Note that it was difficult to obtain good signal-to-noise spectra of cytosine/Au; the band intensities are only about 1/100 of the ones obtained for adenine/Au.

Again, a new band appears in the SER and TER spectra at low wavenumbers which is due to the metal-adsorbate interaction and therefore absent in NR spectra. In comparison to the peak observed in the SER spectrum by Otto *et al.* at 210 cm^{-1},[140] we find an upshift of this band to slightly higher wavenumbers (248 cm^{-1}). A very similar band position, 245 cm^{-1}, has been reported in another SER study on cytosine/Ag,[183] thus we cannot conclude that it is simply the difference in metal causing the upshift, but clearly also the influence of other experimental conditions (sample preparation, laser frequency and power, cleanliness).

The ring breathing mode of cytosine, though strong in the NR spectrum as well as in the SER spectrum, appears only of medium intensity in the TER spectrum. The band position, 785 cm^{-1}, is very close to the one reported for polycrystalline cytosine, 787 cm^{-1}, and differs strongly from the one reported for the SER spectrum, 796 cm^{-1}. According to Sánchez-Cortés and García-Ramos, who carried out a SER study on Ag colloids, the position of the cytosine ring breathing mode strongly depends on the surface coverage. At lower coverages, it appears at 786 cm^{-1}, whereas at higher coverages it is located at around 800 cm^{-1}.[183] As cytosine does not bind very strongly to Au,[173] our rinsing procedure very likely does not only wash off multilayers, but also molecules adsorbed in the first adsorbate layer. Therefore, we expect a very low coverage, which explains the low wavenumber for the ring breathing mode as well as the bad signal-to-noise ratio that turned spectral recording quite difficult for the cytosine/Au sample.

The strongest mode observed in the TER spectrum appears at 1596 cm^{-1} with a shoulder at 1518 cm^{-1} in the C-O stretch region. The double bond character of the C-O vibration, however, seems to be significantly reduced, as deduced from the large downshift in comparison to the 1655 cm^{-1} in the NR spectrum. The SER

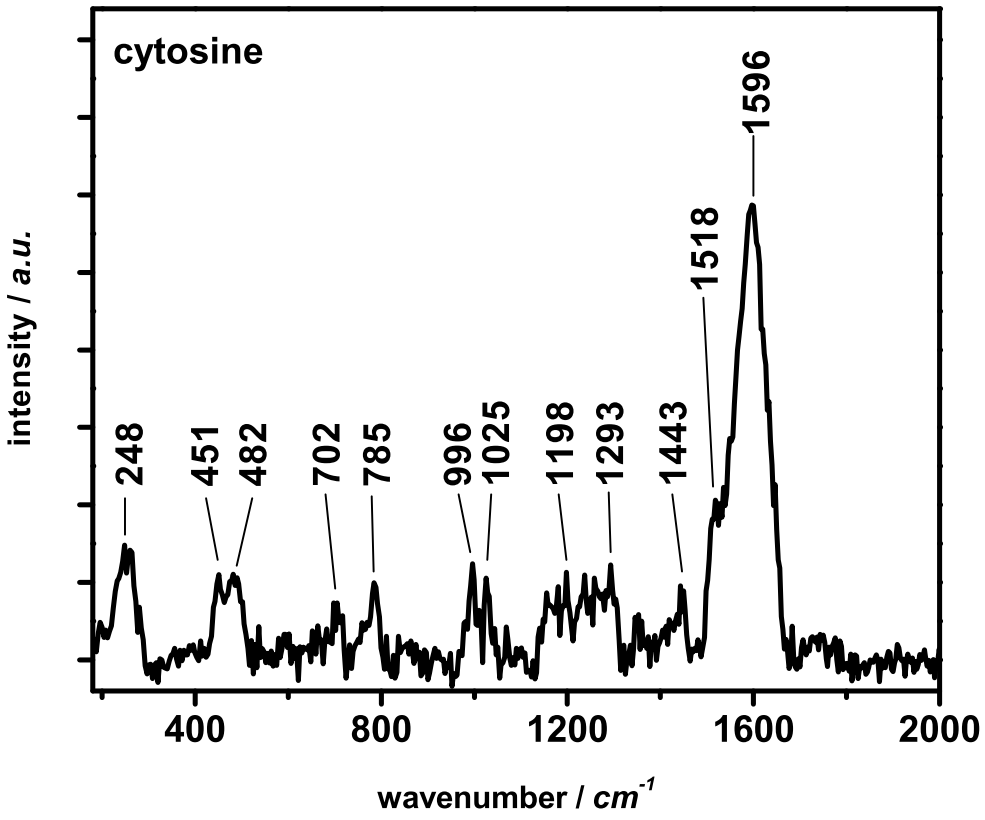

Figure 6.3: TER spectrum (background-corrected) of one (sub)monolayer cytosine adsorbed at Au(111). One strong, broad band is observed at 1596 cm^{-1}, which is assigned to a C=O stretch. The in-plane C=C and C=N stretch modes that appear between 1200-1500 cm^{-1} are of similarly weak intensity as the ring breathing mode (785 cm^{-1}).

spectra report bands at 1640 cm^{-1} (Ref. [140]) or 1625 cm^{-1} (Ref. [183]), which is already a considerable difference to the band position found in the NR spectrum. It is explained by the Ag-adsorbate interaction through the carbonyl oxygen that decreases the double bond character. In our case, however, no other indications of a strong cytosine-Au bond are at hand (the ring breathing band is fairly small in comparison to the C-O band). An alternative explanation that takes into consideration the weak cytosine-Au interactions and the corresponding weakly intense Raman spectra is the change from keto to enol tautomeric form of the molecule upon adsorption. The presence of the metal likely facilitates deprotonation of the nitrogen atom, resulting in protonation of the carbonyl moiety and allowing a weak N-Au interaction. As our current experimental conditions do not allow the determination of the pH at the surface or its potential, we cannot draw any further conclusions from the TER spectra alone.

The in-plane C=N and C=C stretch modes that appear in the region between 1200-1500 cm^{-1} are of similarly weak intensity as the ring breathing mode and support the theory of weak adsorbate-metal interaction. Rasmussen and Deckert recently published an AFM-TER study of cytosine with equally weak signal intensity.[141] Only two Raman bands, the ring breathing at 784 cm^{-1} and a C=C, C=N stretch at 1285 cm^{-1}, appear in the spectrum, in addition to a small bump between 850 - 1000 cm^{-1}. In agreement with our assumption, they rule out any chemical enhancement, i.e. cytosine-Ag interaction, to explain the low-intensity spectral features.

Table 6.2: Assignment of the TER vibrational modes of cytosine (ML) adsorbed at Au(111) at pH 7 according to NR and SER spectra by Otto *et al.*[140]

mode	NRS	SERS (Ag)	TERS (Au)	plane	assignment
1		210 s	248 m	-	metal-cytosine
2		430 w	451 m	in	bend C2-N1-C6, bend N3-N4-C5
3		470 vw	482 m		?
4	546 w	558 m	536 vw	out	bend N1-C2-N3, bend C2-N3-C4
5	598 m	616 w	590 vw	out	bend C2=O, bend N1-R, bend C4-N7
6		690 m	702 w	in/out	bend C5-C4, bend C4-N3, bend N1-C2=O, N3-C2=O
7	787 vs	796 vs	785 m	in	ring breath whole molecule
8	975 w	986 w	996 m	in	bend C5-H
9		1020 m	1025 m	in	rock NH_2, str C2-N3
10	1116 w	1140 vw	1155 w	in	str N1-R, bend C6-H
11			1180 w		?
12		1196 s	1198 m	in	bend C6-H, C4-N7
13	1230 m	1230 m	1237 w		?
14			1258 w		?
15	1294 s	1306 s	1293 m	in	str N1-C6, str C5-C6
16	1368 m	1360 vw	1353 w		?
17	1442 w	br 1422 w	1443 w	in	str C4-N7, N1-C2
18	1536 vw	br 1508 m	1518 s	in	str N3-C4, N1-C2
19		br 1582 m	1596 vs	in	str C4-C5, C5-C6
20	br 1655 s	1640 s		in	str C2=O, str C2-N3

6.2.3 Guanine

The TER spectrum of the second purine nucleobase guanine is shown in Fig. 6.4. Analysis and band assignment are carried out according to Table 6.3, where NR and SER (Ag) data from Giese and McNaughton (Ref. [184]) are listed together with our TER data.

Before analyzing the TER spectrum in detail and comparing it to the NR and SER spectra, attention should be drawn to the fact that the Raman spectral features of guanine vary with the pH of the solution,i.e. the protonation state of the (adsorbed) molecule. In alkaline solutions (pH 11), guanine is present in the deprotonated state, in neutral solution (pH 7) in the neutral state, whereas in acidic solution, its keto form is likely to be protonated.

Band positions from both, guanine SERS spectra (on Ag) recorded at pH 5 and pH 11 are given in Table 6.3 in order to facilitate the comparison and assignment of the TER spectral features. We adsorb from "neutral" ethanolic solution that contains 10^{-3} M guanine - the measured pH of this solution is 11.5. Because of the very low solubility of guanine, we work with saturated solution by using the clear part of the solution for the adsorption ($c << 10^{-3}$). Our experimental conditions do not allow precise control of pH and adsorption state during the experiment, as mentioned before. The resulting complications will manifest themselves during the interpretation of the TER spectrum, as follows.

The Au-guanine bond vibration is found at 223 cm^{-1} (not mentioned in the SERS data at hand, but found in the literature at around 220 cm^{-1}).[140, 185] Compared to the other three DNA bases, this is the lowest wavenumber obtained in our TERS experiments for the base-Au interaction. However, it is known from TDS experiments that guanine adsorbs more strongly on Au than its sister nucleobases.[173] This observation coincides well with the fact that TER spectra with well-pronounced bands were obtained without difficulty from the guanine/Au(111) samples. The signal-to-noise ratio, however, is quite low. Due to the low solubility of guanine, the adsorption solution concentration is reduced in comparison to the other bases' ones. Thorough rinsing, in addition, removes small, undissolved nanocrystals of guanine, additionally lowering the adsorbate concentration.

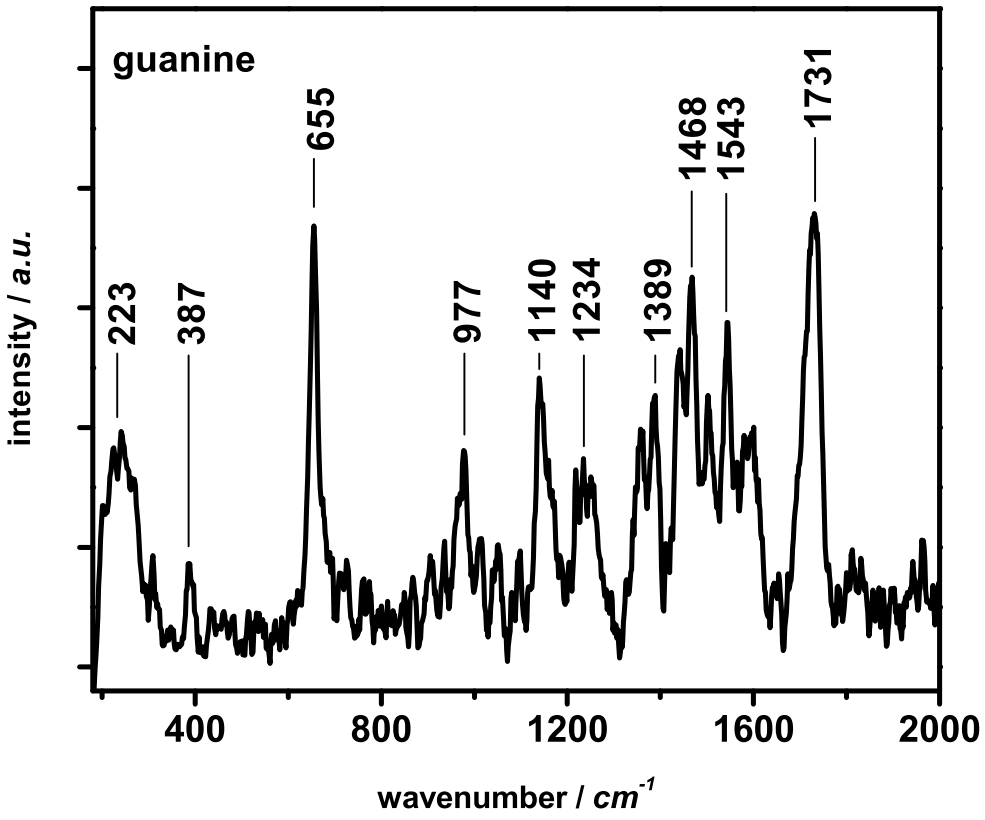

Figure 6.4: TER spectrum (background-corrected) of a (sub)monolayer guanine adsorbed at Au(111). Although the guanine Raman fingerprint is easy to obtain, its signal-to-noise ratio is quite bad. Due to the very low solubility of guanine, the surface concentration is reduced in comparison to the other bases. As a result, vibrational bands that can be assigned to protonated and deprotonated guanine as well as nanocrystals are found in the TERS spectrum (see text for details).

The prominent ring breathing mode of guanine appears at slightly lower wavenumbers in the NR spectrum (647 cm^{-1}) than in the TER and SER spectra (655 cm^{-1}). This is ascribed to the influence of the present metal surface. Even a slightly larger shift to 659 cm^{-1} has been reported in the literature.[185]

Note that, in contrast to adenine and cytosine, only in-plane modes are present in the TER spectrum. As we assume a rather strong interaction between guanine and Au, this lack of out-of-plane modes hints to the conclusion that the molecule is adsorbed mainly upright at the metal surface.

The ring deformation modes that appear at 387 cm^{-1} and 977 cm^{-1} in the TER spectrum are both shifted upwards with respect to the band positions in the NR and SER spectra, in contrast to the NH_2 rocking band at 1140 cm^{-1} (SER and TER alike) which is found at much higher wavenumbers in the NR spectrum. Both ring and external amino group seem to be strongly influenced by the presence of the metal, although it appears that the NH_2 rocking vibration is not as sensitive to the kind of metal substrate (Au or Ag, smooth or rough). The ring deformations mainly involve the π-electron system and thus are more strongly influenced by the guanine-metal bond, which is likely to take place via one of the ring nitrogens.[184, 186] Therefore, they are more sensitive to the substrate properties.

A strong TER band is found at an extremely high wavenumber, 1731 cm^{-1}. Although the corresponding SER band is found slightly lower at 1710 cm^{-1}, it is nevertheless mainly assigned to the C=O stretching mode. The strong upshift in comparison to the NR band position (around 1680 cm^{-1}) has been predicted theoretically to reach to 1734 cm^{-1},[184] very close to the band position found in the TER spectrum. A strong interaction between guanine and the metal is likely to be the cause for this upshift. Only a significant alteration of the electronic structure in the ring will have a strong influence on the carbonyl stretching mode, the upshift indicating a strengthening of the C=O double bond character.

The majority of the TER bands in the guanine spectrum appear between 1100-1600 cm^{-1}. They are assigned to C=C and C=N stretching modes, the NH_2 scissoring mode and some C-H and N-H bending modes. Interestingly, the assignment is not as straightforward as one could expect from a strongly adsorbed DNA base. Bands that appear in the TER spectrum (adsorbed from a pH 11 saturated solution) show

similarities to NR and SER bands of pH 11 and 5 alike. The ratio of spectral features for pH 5- to pH 11-"matches" is 2:1. This suggests that both, protonated and deprotonated species are adsorbed at the surface in the sampling area, although, regarding the pH of the solution, one would not expect neutral guanine to be present. In the TER spectrum, we find as many bands that can be assigned according to the SERS data as according to the NR data. This can be explained by the fact that we employ a saturated guanine solution: Taking out clear solution for the adsorption from the saturated solution beaker cannot be performed without dragging along tiny guanine crystals. These, naturally, adhere to the surface, too, and add to the guanine TER spectrum, exhibiting typical NR features from polycrystalline guanine.

Table 6.3: Assignment of the TER vibrational modes of guanine (ML) adsorbed at Au(111) at pH 7 according to NR and SER spectra at pH 11 (pH 5) by Giese and McNaughton.[184]

mode	NRS	SERS (Ag)	TERS (Au)	plane	assignment
1			223 m		
2	340 vw (340 vw)[a]	br 361 vw (362 vw)	387 w	in	def R (sqz group N7-C5-C6-O
3	647 s (649 vs)	653 vs (647 vs)	655 vs	in	breath R6
4	963 w (937 m)	956 w (954 m)	977 m	in	def R5 (sqz N7-C8-N9
5	1193 m (1186 vw)	1141 w (1135 w)	1140 m	in	rock NH_2, str C6-N1
6	1224 m (1234 s)	1220 w (br 1214 m)	1234 w	in	bend C8-H, str C5-N7-C8
7	1266 vs (1265 m)	1273 m (1261 vw)	1250 w	in	str C5-N7, C4-N9, C5-C6, C6-N1, C2-N3
8	1324 m (1361 w)	1328 m (1359 w)	1357 m	in	bend N1-H, N10-H12, str C2-N10
9	1371 m (1390 m)	1381 m (1385 m)	1389 m	in	str C4-C5, C4-N9, N7-C8, N1-C2, rock NH_2
10	(1421 w)	br 1448 m	1442 m	in	bend N9-H, str C8-N9, N3-N4, N1-C2
11	1456 s (1468 vw)	br 1461 m (1461 m)	1468 s	in	str N1-C2, N3-C4, N7-C8, bend C8-H
12	1503 w		1502 m	in	sciss NH_2, str C2-N3
13	1537 s (1551 m)	(1532 w)	1543 s	in	str N3-C4, bend N9-H
14		(1588 vw)	1580 m	in	sciss NH_2, str C2-N10
15	(1602 vw)		1600 m	in	sciss NH_2, str C2-N10
16		(br 1710 s)	1731 vs	in	str C6-O, C5-C6, bend N1-H

[a] pH 11 (pH 5)

6.2.4 Thymine

In Fig. 6.5, the TER spectrum of thymine on Au(111) is presented. Analysis and assignment are performed according to NR and SER spectra presented by Cunha *et al.*[187] The cited study clarifies discrepancies encountered in the literature on thymine SERS studies, because it presents potential-dependent spectra. In our experiments, we work at open circuit potential (OCP) at around -0.1 V vs Ag/Ag^+.[1] Comparing our TER spectrum to the SER spectrum by Cunha *et al.* recorded at -0.1 V vs Ag/Ag^+,[187] an astonishing similarity of the spectral features is found. This confirms our OCP values and allows us to follow the authors' conclusion of a strongly tilted adsorption geometry of thymine at the metal surface in this potential region, which has, in fact, been proposed also by other groups.[188–190]

It was quite difficult to obtain a reasonable TER spectrum of thymine at Au(111). Only very few bands are detected, and the characteristic ones are of very weak intensity. The thymine-metal bond vibration is found at 257 cm^{-1} in the TER spectrum. This band position is extremely upshifted in comparison to the one observed by Cunha and coworkers (216 cm^{-1}). A thorough investigation by Cho *et al.* about the SER spectral differences between thymine/Ag and thymine/Au systems showed that, indeed, the band position is largely upshifted in the case of gold to 259 cm^{-1}, which coincides perfectly with our value.

The fact that we mainly observe out-of-plane modes supports the idea of a strongly inclined molecular orientation. Bands assigned to the bending of C=O (651 cm^{-1}), the torsion (1177 cm^{-1}) and the deformation (1423 cm^{-1}) of the methyl group are strong indicators for a flat adsorption geometry. They are only very weakly, if at all, present in the NR spectrum, and visualization is possible only through the extreme large field enhancement, if the vibrations are aligned with the near-field.

The most intense feature always present is the broad band at around 1600 cm^{-1}, with a shoulder at 1571 cm^{-1}. This peak is assigned to in-plane C=O stretch modes. In general, a strong C=O double bond would be expected at somewhat higher wavenumbers. Therefore, the band position agrees well with the concept that thymine is bound via the carbonyl group(s) at low potentials, which lowers the C=O

[1] OCP measurements were carried out in the laboratory of Constanze Donner, Freie Universität Berlin.

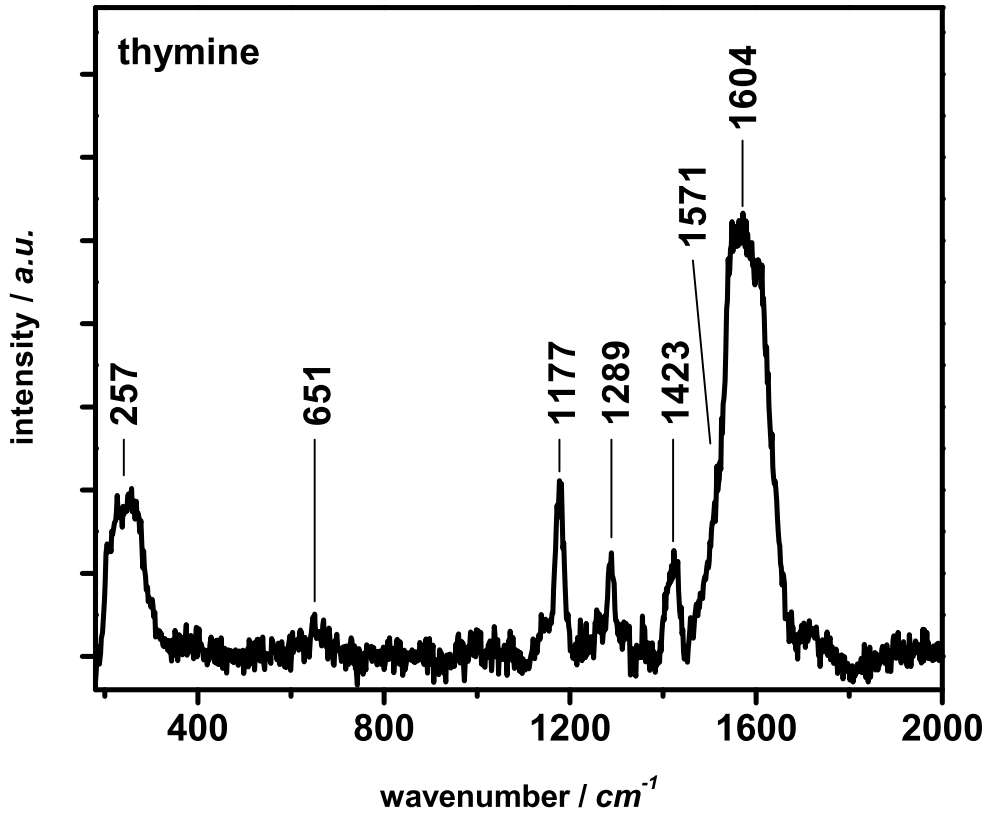

Figure 6.5: TER spectrum (background-corrected) of a (sub)monolayer thymine adsorbed at Au(111). The appearance of out-of-plane modes (1177 cm^{-1} and 1423 cm^{-1}) in combination with the strong C=O stretch mode at 1604 cm^{-1} (the double bond bond is weakened by O-Au interactions) points to a rather flat adsorption geometry of thymine at Au(111) upon our experimental conditions, in agreement with the literature.

bond strength. Bonding through the oxygen lone pairs to the surface perfectly enables the molecule to adapt a quite horizontal orientation, as they stick out of the molecular plane.

The second in-plane mode is observed at 1423 cm^{-1}. It is assigned to a ring stretching vibration. Its low intensity is due to the large tilt of the molecule with respect to the surface normal. The in-plane ring breathing mode, which is very prominent at 783 cm^{-1} (740 cm^{-1} for solid thymine) in many SERS publications, is absent in the recorded TER spectrum. Cunha *et al.* find this Raman band only at positive electrode potentials.[187] In agreement with our results, their spectrum at -0.1 V vs Ag/Ag^+ does not exhibit this spectral feature. The authors conclude that, the lower the potential, the more inclined with respect to the surface normal the molecule is adsorbed.

As a result of the analysis of the TER spectrum of thymine at Au(111), following the argumentation of Cunha *et al.*, we arrive at the conclusion that upon our experimental conditions, working at an OCP of around -0.1 V vs Ag/Ag^+, thymine is adsorbed at the Au via the carbonyl group(s) and adapts a nearly horizontal orientation at the surface.

Table 6.4: Assignment of the TER vibrational modes of thymine (ML) adsorbed at Au(111) at pH 7 according to NR and SER spectra by Cunha *et al.*[187]

mode	NRS	SERS (Ag)	TERS (Au)	plane	assignment
1		216 m	br 257	-	metal-thymine
2	615 m	633 m	651 vw	out	bend C=O
3	1155 w	1178 w	1177 m	out	tors CH_3
4	1250 w	1220 vw	1222 vw	in (out)	def ring C6-N1 (str CH_3)
5		1290 vw	1289 w	in	str ring[a]
6		br 1440 w	1423 w	out	def CH_3
7		1585 s	sh 1571 m	in	str C=O
8		sh 1639 vw	br 1604 s	in	str C=O

[a] Assignment according to Ref. [191]

6.3 Conclusions, difficulties & outlook

We have shown that Raman spectra of smallest amounts of nonresonant nucleobases adsorbed on atomically smooth Au(111) can be obtained with TERS. The characteristic vibrational fingerprint of each DNA base allows the straightforward identification of the molecule down to 0.8 nmol/cm^2. With these results, we have proven that TERS is a highly sensitive analytical tool that can be employed also for nonresonant species, widening its application possibilities to biochemically or biophysically relevant species. This elementary study will open up routes towards the label-free investigation of nucleosides or whole DNA strands.

The interpretation of the spectra with respect to the molecule-metal interaction suggests that adenine is adsorbed strongly on Au(111) upon our experimental conditions. Cytosine seems to be more weakly bound, supposedly in its enol form, to the surface. Guanine samples were difficult to prepare from saturated solution because of the base's low solubility. As a result, deprotonated and protonated guanine, as well as nanocrystals are identified in the TER spectrum. A different preparation method should overcome this problem. The TER spectrum of thymine shows spectral characteristica of a flat-lying adsorbate. In agreement with the literature, thymine binds to gold with its oxygen atoms rather horizontally at the surface upon our working conditions.

Note that upon the given experimental conditions, the nucleobases adsorb at the open circuit potential (OCP). This simply means that we do not control the Au electrode potential during adsorption. As we derived from experiments performed in the laboratory of Constanze Donner at the Freie Universität Berlin, the OCP strongly depends on the solution concentration and the influence of oxygen and present anions, and of course also on the metal substrate. The variety of guanine adsorption states detectable in the TER spectra clearly demonstrates the main difficulty: the preparation method and control of the self-assembly of the nucleobases at the gold surface. Adsorbing the target molecules under potential control in an electrochemical cell might improve the adsorption procedure. It may be possible to form well-defined monolayers that are stable enough to transfer the sample to the TERS set-up and allow measurements in air for some time before degradation. We are currently construct-

ing an electrochemical TERS set-up (see Chapter 7). Working upon electrochemical conditions at the metal-liquid interface will provide us with the advantage of a controlled surface potential, solution concentration, pH and cleanliness during the TERS experiment, facilitating defined adsorption and maybe even allowing an orientation analysis of the nucleobases at Au(111).

In order to better understand the interaction between the nucleobases and the Au(111) substrate, a systematic study of the pH of the adsorption solution and of the solvent are carried out for adenine and guanine. Further work in progress includes the investigation of the coadsorption of adenine and thymine at Au(111), two complimentary DNA bases, by TERS and subtractively normalized Fourier transform infrared spectroscopy (SNFTIRS), a first step towards the study of DNA strands.