Revealing DNA Interactions with Exogenous Agents by Surface-Enhanced Raman Scattering

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ABSTRACT: The standard protocols for DNA analysis largely involve polymerase chain reaction (PCR). However, DNA structures bound to chemical agents cannot be PCR-amplified, and therefore any sequence changes induced by external agents may be neglected. Thus, the development of analytical tools capable of characterizing the biochemical mechanisms associated with chemically induced DNA damage is demanded for the rational design of more effective chemotherapy drugs, understanding the mode of actions of carcinogenic chemicals, and monitoring the genotypic toxicology of environments. Here we report a fast, high-throughput, low-cost method for the characterization and quantitative recognition of DNA interactions with exogenous agents based on surface-enhanced Raman scattering spectroscopy. As representative chemical agents, we selected a chemotherapeutic drug (cisplatin) which forms covalent adducts with DNA, a duplex intercalating agent (methylene blue), and a cytotoxic metal ion (HgII) which inserts into T:T mismatches. Rich structural information on the DNA complex architecture and properties is provided by the unique changes of their SERS spectra, which also offer an efficient analytical tool to quantify the extent of such binding.

INTRODUCTION

Genomic stability is continuously threatened by damages induced by both endogenous factors associated with physiological processes and exogenous agents, e.g., ionizing radiations such as UV light, environmental toxic agents such as industrial chemicals or smoke, chemotherapy drugs, etc.1–4 To respond to these threats, cells have evolved an intricate series of interlocking mechanisms to maintain their genomic integrity.1,4 However, failures in DNA repair can occur, leading to increasingly high mutation rates or wider scale genomic aberrations that fuel the development of cancer.2,3 On the other hand, DNA-damaging agents that impair base-pairing and/or block DNA replication and transcription have evolved as one of the most effective classes of compounds in cancer therapy.3,5 Furthermore, the doping of metal ions into DNA duplexes gives rise to an exciting area of research in nanotechnology, allowing for the design of molecular electric circuits in which the outstanding properties of DNA for bottom-up engineering of rationally designed self-assembled two- and three-dimensional molecular architectures are combined with metal ions in hybrid materials to give enhanced conductivity or magnetic properties, as well as other interesting electronic effects.6–8 Therefore, development of analytical procedures capable of in-depth characterization of the biochemical mechanisms associated with chemically induced DNA changes is of paramount importance to unravel the action of chemicals that cause genotypic damages including cancer, trace exposure to dangerous environments, rationally design new chemotherapy drugs with a more effective mechanism of action, and develop chemical strategies to circumvent specific drug resistance mechanisms.6 Similarly, a full understanding of the structural and electronic modifications of the duplex structures upon metal ion binding is also key for future engineering and fine-tuning of related nanomaterials and nanodevices.6–8

A large number of different and often case-specific strategies have been developed in order to detect and elucidate the interaction mechanisms of drugs, toxic agents, and metal ions with DNA, such as classical ultraviolet–visible (UV–vis) spectroscopy,7,10,11 fluorescence and infrared (IR)/Raman spectroscopies,10,12 mass spectrometry (MS)-based approaches,7,13 nuclear magnetic resonance (NMR) spectroscopy,14 linear and circular dichroism (CD),7,11,12 and electrochemical methods.12 However, for different reasons, none of these techniques alone fully addresses the requirements in terms of sensitivity, versatility, cost-effectiveness, and simplicity. Importantly, when it comes to sensitivity, it is worth...
highlighting that DNA structures covalently bound to chemical agents, such as platinum-based anticancer drugs\textsuperscript{15,16} or photoactive dyes,\textsuperscript{17} cannot be amplified via polymerase chain reaction (PCR). Similarly, intercalating molecules may result in an inhibition of PCR amplification.\textsuperscript{18} Therefore, DNA sequence changes induced by external agents usually remain undetected.

Plasmonic-based biosensing has emerged as a very efficient strategy for the development of highly selective, prompt, low-cost, and user-friendly optical DNA sensors,\textsuperscript{19–22} overcoming the intrinsic low throughput and high cost of analytical procedures such as PCR and DNA microarray techniques. In particular, direct label-free surface-enhanced Raman scattering (SERS) analysis of DNA has shown an outstanding analytical potential, with the ability to provide detailed chemical information on the sequence structure and its modifications.\textsuperscript{23–31} However, the final SERS of a complex biomolecule such as DNA is highly sensitive to a large set of interdependent multiple factors and experimental variables that are difficult to control and often case specific. As a result, the acquisition of reproducible SERS spectra at low DNA concentrations has usually represented a major challenge, especially in the case of double-stranded structures (dsDNA) where the negatively charged phosphate backbone (at the physiological pH) shields the direct interaction of the nucleobase with the metal surface.\textsuperscript{32,33} Therefore, control at the biomolecular level of the chemical interaction of DNA and metal is likely the key point toward the improvement of spectral reproducibility, since the final molecular conformation and orientation adopted by the strand on the plasmonic surface, as well as the binding specificity for the metal, strongly influence the final SERS spectrum. An elegant strategy to tackle such challenges was recently reported.\textsuperscript{30,31} Highly reproducible SERS spectra of genomic DNA were acquired via physical entrapment of the duplex within the interparticle volume of highly concentrated citrate-capped silver colloids, allowing real-time monitoring of radical-induced DNA damages. However, that strategy proved to be effective only at a relatively high DNA concentration, i.e., 1 mg/mL.\textsuperscript{29}

Another alternative implies the use of capping ligands with the ability of interacting with nucleic acids. Among others, spermine (Sp) is known to electrostatically interact with the phosphate groups of DNA\textsuperscript{34} and has been proven to act as a stabilizing ligand for silver colloids, conferring upon them positive charge.\textsuperscript{35} Thus, addition of negatively charged DNA sequences to spermine-coated silver nanoparticles (AgNP@Sp) promotes their fast aggregation into long-term stable clusters in suspension where the DNA strands remain trapped at the interparticle junctions.\textsuperscript{31} Herein, we exploit AgNP@Sp colloids in the direct ultrasensitive SERS analysis of dsDNA complexes with exogenous agents, including both short synthetic oligonucleotides and real genomic DNA, at nanogram levels. Specifically, we demonstrate the direct SERS detection of DNA in its native state when complexed with three chemicals representative of different molecular classes of exogenous agents: (i) a chemotherapeutic drug, cisplatin (CP), which forms covalent adducts with DNA,\textsuperscript{36} (ii) an organic dye, methylene blue (MB), which intercalates into the space between two adjacent base pairs of the DNA duplex,\textsuperscript{37} and (iii) a metal ion, Hg\textsuperscript{II}, which inserts into T:T mismatches.\textsuperscript{38} Binding of duplexes with these exogenous agents is revealed by the unique and characteristic vibrational alterations of the SERS spectra, which are also quantitatively correlated with the number of binding events per duplex. This low-cost, high-throughput, and flexible method finally combines the intrinsic sensitivity of the SERS phenomenon (i.e., no need for DNA preamplification steps) with the reliable, reproducible, and rich structural information provided by the Raman signatures of very different classes of DNA complexes. We believe that this novel SERS-based method provides a powerful but simple analytical tool for investigating and quantifying the interactions of DNA with, potentially, all classes of known and yet unknown exogenous agents.

**RESULTS AND DISCUSSION**

AgNP@Sp colloids were prepared by direct reduction of Ag\textsuperscript{+} ions with sodium borohydride in aqueous solution in the presence of spermine hydrochloride,\textsuperscript{35} yielding stable, positively charged spherical nanoparticles (Figure 1A) with minimal SERS background (Figure S1).

The dsDNA:NP ratio requires optimization in order to generate clusters with long-term stability in dispersion providing intense, unvaried, and reproducible averaged bulk SERS spectra with well-defined average centers, bandwidths, and relative intensities (Figure S2).\textsuperscript{31,39} This condition, in the case of short double-helix sequences (ds1 and ds2, 21 base pairs, see Figure 1), is satisfied in the ca. 0.3–6.3 μg/mL concentration range,\textsuperscript{31} whereas for the long genomic structure of dsDNA from the thymus of calves (CTds), here selected as a model of real DNA, the optimum concentration lies in the interval between ca. 4 and 26 μg/mL (Figure S3). Thus, as it should be reasonably expected, major differences in DNA length have some degree of influence on the nanoparticle aggregation pattern (cluster size and geometry distribution, average interparticle distances, etc.). We therefore fixed the short synthetic duplexes (ds1 and ds2) and genomic CTds concentrations at 1.3 and 7.8 μg/mL, respectively, throughout the whole SERS analysis. Differential centrifugal sedimentation (DCS) analysis was used to investigate both nanoparticle diameter and DNA-induced aggregation patterns. DCS technique is increasingly used for high-resolution nanoparticle sizing due to its ability to resolve small aggregates from monodisperse particle populations present in dispersion.\textsuperscript{22,40} Direct sizing and detection of aggregation patterns in dispersion overcome the obvious limitations associated with drying artifacts often observed when using TEM. Figure 1A,B shows an overlay of the extinction spectra and DCS analysis of the DNA-induced aggregates, respectively. In both cases, ds1 and CTds promote aggregation of the individual AgNP@Sp nanoparticles into highly stable clusters of relatively homogeneous size distribution, centered respectively at 109.1 and 114.3 nm, as measured by DCS. In particular, a significantly narrower distribution is obtained in the case of the short duplex (Figure 1B and Figure S4).

On the other hand, the SERS spectra of short synthetic and genomic dsDNAs (Figure 1C) do not show any significant variations in average band centers and bandwidths, whereas minor changes in relative band intensities are associated with the different base composition.\textsuperscript{31} This result represents an extremely important piece of evidence on the reliability and solidity of the sensing strategy to investigate DNA sequences, even for extreme differences in biomolecule size.

It is important to stress that SERS measurements are performed under an *averaged bulk SERS regime*\textsuperscript{39} using a long working distance objective to investigate the stable DNA-mediated aggregates in suspension. Furthermore, the samples
were measured after 2 h incubation with the DNA, when conformational equilibrium of the DNA onto the metal was achieved. Under these conditions, hypothetical fluctuations in the SERS spectra associated with local differences in DNA adhesion events are completely averaged out. Conversely, the scattering volume investigated by the laser containing the nanoparticle/DNA clusters that contribute to the final SERS spectra is much less than the actual sample volume (100 μL).

**Formation of DNA Adducts by Cisplatin.** Since the initial discovery of its anticancer activity, CP (and its analogues) combination chemotherapy has represented the cornerstone of treatment of many cancers. The inorganic compound CP forms covalent adducts with DNA, the most prevalent of which (>80%) is the 1,2-intrastrand cross-link between neighboring purine bases (preferably guanine via binding to the N7 atom). Such chemical binding leads to a large distortion of the duplex and loss of helical stability that ultimately triggers cell apoptosis. Despite its great efficacy at treating specific kinds of cancers, CP suffers from several side effects and intrinsic limitations (such as acquired resistance of cells to the drug) which have fueled an extensive amount of research aiming to develop new platinum-based drugs. However, only very few of these drug candidates have succeeded in entering clinical trials, possibly because their mechanism of action was neither fully understood nor reproducibly used as the basis for their chemical design. Importantly, the cell resistance to CP-based chemotherapy remains poorly understood, even though it has been demonstrated that it is directly related to the extent of DNA damage.

SERS monitoring of the CP binding to DNA was restricted so far to thiolated sequences immobilized on gold nanoshells, where a simple reduction in SERS spectral reproducibility was observed upon the addition of the chemotherapeutic drug. Differently, when AgNP@Sp colloids are employed as plasmonic substrates, the deformation of the duplex induced by CP covalent binding is clearly and reproducibly reflected in the characteristic alteration of the SERS signal. Figure 2A shows...
the SERS spectra of the 21-base-pair duplex (ds1) and the corresponding adduct with CP (ds1CP), as well as the digitally calculated ds1CP − ds1 difference spectrum. Subtraction of the reference SERS spectrum of ds1 from that of the adduct yields a difference spectrum that fully reveals the vibrational signatures associated with the complexation. Characteristic spectral features of the covalent adduct formation mainly lie in the 1300−1600 cm⁻¹ region, such as the informative intensity decrease of the 1485 cm⁻¹ band, which has been associated with the binding of electrophilic agents to the N7 atom.44 Moreover, the marked intensity increase of the guanine contributions (ca. 1333 and ca. 1588 cm⁻¹), combined with the weakening of the guanine C=O stretching (1726 cm⁻¹) and the simultaneous enhanced intensity of the carbonyl contribution at 1665 cm⁻¹, has been associated with DNA premelting and/or denaturation at the guanine residues.45,46 In addition to these spectral changes, it is also worth noting alterations of the SERS spectra at lower wavenumbers, such as the intensity decrease and red-shift of the A and C+T ring breathing modes, which are also consistent with the distortion of the duplex and its partial unwinding. The appearance of a new broad, intense feature at ca. 541 cm⁻¹, which is ascribed to the Pt-NH3 stretching of CP ligands,47 was also observed. The presence of this band can be associated with both CP molecules involved in the DNA complex as well as the direct adsorption of free CP molecules onto the metal surface (Figure S1). Such characteristic spectral “fingerprints” of the CP adduct not only selectively inform about the type of complexation but can be also quantitatively correlated to the extent of such interactions. Figure 2B illustrates SERS spectra in the 1430−1650 cm⁻¹ region obtained in the presence of increasing RCP = [CP]/[ds1] molar ratios (the ds1 concentration was kept fixed throughout the whole study). These spectral changes are correlated quantitatively with CP concentration using as spectral marker the ratiometric peak intensity, I₁₅₈₆/I₄₈₆₀ which is plotted against RCP in Figure 2C. Linear correlation (r² > 0.98) is observed in the whole investigated range of RCP = 0−50, with a detection limit close to the equimolar ratio (corresponding to 1 CP molecule per 21 base pairs).

**Intercalation of the Organic Dye Methylene Blue into the Duplex.** The interaction of small ligand molecules with DNA sequences takes place also in a non-covalent fashion, e.g., via intercalation of planar aromatic molecules into the space between two adjacent base pairs.57 The insertion of the DNA intercalating chemicals generally induces local structural changes to the DNA, including unwinding of the double helix and lengthening of the strand, which may lead to genotoxic effects such as, for instance, frameshift mutagenesis.57 Methylene blue belongs to the class of phenothiazinium dyes, and it has been employed in photodynamic therapy of tumors and other diseases.48 Additionally, MB has been also been exploited in antimicrobial chemotherapy, particularly in the area of antimalariais,49 and as a DNA staining agent.50 Previous studies indicate that MB mainly binds dsDNA via intercalation of its aromatic moiety, whereas the positive charge of MB would improve the DNA binding affinity by electrostatically interacting with the phosphate groups.50

In contrast to CP, MB is an aromatic molecule with high Raman cross-section providing an intense SERS spectrum. In fact, the new intense features arising in the spectrum of the equimolar ds1MB complex are ascribed to the dye contribution, which largely dominates the corresponding difference spectrum (ds1MB-ds1, Figure 3A). Importantly, the SERS profile of the intercalated MB significantly differs from that of the molecule directly adsorbed onto the colloidal nanoparticles (Figure 3A), as revealed by the 3 nm upshift of the strong C–C ring stretching (1624 cm⁻¹),51 the remarkable spectral reshaping in between 1380 and 1520 cm⁻¹ (including the band at 1430 cm⁻¹, ascribed to the asymmetric C–N stretching52 and the C–C stretching of the ring52 at 1475 and 1503 cm⁻¹), and the sharp band at 1038 cm⁻¹ associated with in-plane bending CH vibrations.51 This spectral reshaping is associated with the specific electronic perturbation of the MB structure upon insertion within the aromatic nucleobases. Interestingly, when MB is left to interact with single-stranded sequences, the observed SERS spectra of the mixture show MB contributions that largely match those of the pure SERS of the intercalating agent (Figure S6). This spectroscopic evidence clearly suggests that MB molecules that loosely bind ssDNA via weak electrostatic interactions53 are free to directly interact with the metal surface, producing the characteristic SERS signal of the MB-Ag surface complex. In contrast, in the presence of dsDNA, the effective sequestration of the MB molecules in the helix, sandwiched between aromatic heterocyclic base pairs by π−π stacking and dipole–dipole interactions, leads to a markedly different SERS profile. In this case, due to the strong and complex Raman spectrum of MB, a reliable spectral analysis of the binding agent-induced perturbations on the ds1 sequences can no longer be performed by simple difference methods due to the large spectral overlap.
As for CP, we also monitored the SERS response of ds1+MB mixtures at different molar ratios by fixing the ds1 concentration and varying the MB amount (Figure 3B). As a spectral marker, we identified the ratiometric peak intensity \( I_{1624}/I_{1575} \) between the intense MB band at 1624 cm\(^{-1}\) and the ds1 band at 1575 cm\(^{-1}\), which was plotted against the \( R_{\text{MB}} = [\text{MB}]/[\text{ds1}] \) molar ratios (Figure 3C), revealing an excellent linear correlation \((r^2 > 0.99)\) with a limit of detection below \( R_{\text{MB}} = 1 \) (corresponding to ca. 1 dye molecule per 21 base pairs).

**DNA–Metal Ion Coordination: Formation of T-Hg\(^{II}\)-T Base Pairs.** Pyrimidine mismatched base pairs in DNA duplexes are known to selectively capture metal ions to form metal-ion-mediated base pairs.\(^{38} \) In particular, T:T mismatched duplexes are known to selectively capture metal ions to form covalent N3 of the thymine bases is followed by the formation of strong T pairs, a process wherein the dissociation of the imino protons of the thymine bases is facilitated by the formation of strong covalent N3–Hg bonds bridging the opposite pyridiminic bases.\(^{38} \) The formation of T-Hg\(^{II}\)-T pairs within cells is also dependent on the deprotonation of the thymine N3 atom,\(^{59} \) the intensity change of the thymine primarily match those observed for ds2 and are consistent with the ratiometric peak intensities \( I_{778}/I_{793} \) as a spectral marker. The concentration of ds2 was kept constant at 1.3 \( \mu \)g/mL, whereas the Hg\(^{II}\) amount was modified accordingly. (C) Ratiometric peak intensities \( I_{778}/I_{793} \) vs \( R_{\text{Hg}} \) molar ratio in logarithmic scale. Inset: Outline of the Hg\(^{II}\) insertion into the T:T mismatch via binding of the N3 atoms.

Figure 4. (A) SERS spectra of ds2 and the ds2Hg\(^{II}\) mixture (\( R_{\text{Hg}} = [\text{Hg}^{\text{II}}]/[\text{ds2}] = 5 \)), and the corresponding difference spectrum ds2Hg\(^{II}\) – ds2. (B) Detail of the 750–840 cm\(^{-1}\) spectral region for the SERS spectra of ds2Hg\(^{II}\) mixtures at different molar ratios \( R_{\text{Hg}} \) (top to bottom: 0.1, 0.05:1, 0.1:1, 0.5:1, 1:1, and 5:1, corresponding to metal–T:T base pair ratios 0, 0.025, 0.05, 0.25, 0.5, and 2.5, respectively). The concentration of ds2 was kept constant at 1.3 \( \mu \)g/mL, whereas the Hg\(^{II}\) amount was modified accordingly. (C) Ratiometric peak intensities \( I_{778}/I_{793} \) vs \( R_{\text{Hg}} \) molar ratio in logarithmic scale. Inset: Outline of the Hg\(^{II}\) insertion into the T:T mismatch via binding of the N3 atoms.

The SERS spectra of the ds2 heteroduplex, containing two T:T mismatch pairs before and after being exposed to an equimolar amount of HgCl\(_2\), are shown in Figure 4A. Analogously to what observed for the normal Raman study of T-Hg\(^{II}\)-T complex formation,\(^{37,58} \) the intensity change of the ring-breathing band at 785 cm\(^{-1}\) is also consistent with the thymine–metal binding. Insertion of the mercury into the T:T mismatch pocket has also a direct influence on the neighboring base pairs,\(^{58} \) as indicated by the significant change in the relative intensities between the adenine band at 1508 cm\(^{-1}\) and the cytosine feature at ca. 1528 cm\(^{-1}\) (Figure 4A). As an experimental control, we performed the identical SERS study by replacing ds2 with the homopolymeric thymine sequence, pT (Figure S7). The spectral changes revealed in this latter case largely match those observed for ds2 and are consistent with the thymine–metal binding.\(^{37,58} \) On the contrary, no changes in the SERS spectrum were observed when the ds2 heteroduplex was replaced with the fully complementary ds1 (Figure S8).

The spectral changes illustrated in Figure 4B were correlated quantitatively with the molar ratio \( R_{\text{Hg}} = [\text{Hg}^{\text{II}}]/[\text{ds2}] \) using the ratiometric peak intensities \( I_{778}/I_{793} \) as a spectral marker. The plot of \( I_{778}/I_{793} \) vs \( R_{\text{Hg}} \) (logarithmic scale) shows a detection limit of \( R_{\text{Hg}} \approx 0.05 \) (corresponding to one Hg\(^{II}\) ion per 10 T:T mismatch pairs), and \( r^2 > 0.93 \) in the 0.01–5 molar ratio range (Figure 4C). A saturation point is approximately observed for \( R_{\text{Hg}} \geq 2 \) (Figure S9), i.e., when the number of Hg\(^{II}\) ions is equal to or larger than the number of T:T mismatch pairs available. Interestingly, in the case of Hg\(^{II}\) binding, the extent of the spectral changes is not linearly correlated with the number of binding events (i.e., number of Hg\(^{II}\) molecules per duplex) as for CP and MB complexation. It has been shown that the insertion of Hg\(^{II}\) into short DNA duplexes containing multiple T:T mismatches is not affected by pre-existing T-Hg\(^{II}\)-T pairs at different positions within duplex.\(^{11} \) Therefore, we cannot ascribe the logarithmic correlation illustrated in Figure 4C to a decrease of Hg\(^{II}\) binding affinity when the T:T mismatches are progressively saturated within the ds2 duplexes. On the other hand, CD studies indicated that Hg\(^{II}\) binding to dsDNA leads to a progressive conformational transition from B to Z-like structures.\(^{60} \) In this regard, Raman conformation markers of the tertiary arrangement of DNA double helix\(^{61} \) in the SERS spectrum were observed when the ds2 heteroduplex was replaced with the fully complementary ds1 (Figure 8).

The spectral changes illustrated in Figure 4B were correlated quantitatively with the molar ratio \( R_{\text{Hg}} = [\text{Hg}^{\text{II}}]/[\text{ds2}] \) using the ratiometric peak intensities \( I_{778}/I_{793} \) as a spectral marker. The plot of \( I_{778}/I_{793} \) vs \( R_{\text{Hg}} \) (logarithmic scale) shows a detection limit of \( R_{\text{Hg}} \approx 0.05 \) (corresponding to one Hg\(^{II}\) ion per 10 T:T mismatch pairs), and \( r^2 > 0.93 \) in the 0.01–5 molar ratio range (Figure 4C). A saturation point is approximately observed for \( R_{\text{Hg}} \geq 2 \) (Figure S9), i.e., when the number of Hg\(^{II}\) ions is equal to or larger than the number of T:T mismatch pairs available. Interestingly, in the case of Hg\(^{II}\) binding, the extent of the spectral changes is not linearly correlated with the number of binding events (i.e., number of Hg\(^{II}\) molecules per duplex) as for CP and MB complexation. It has been shown that the insertion of Hg\(^{II}\) into short DNA duplexes containing multiple T:T mismatches is not affected by pre-existing T-Hg\(^{II}\)-T pairs at different positions within duplex.\(^{11} \) Therefore, we cannot ascribe the logarithmic correlation illustrated in Figure 4C to a decrease of Hg\(^{II}\) binding affinity when the T:T mismatches are progressively saturated within the ds2 duplexes. On the other hand, CD studies indicated that Hg\(^{II}\) binding to dsDNA leads to a progressive conformational transition from B to Z-like structures.\(^{60} \) In this regard, Raman conformation markers of the tertiary arrangement of DNA double helix\(^{61} \) in the SERS difference spectra are too weak and poorly resolved to extract fruitful information. However, it is reasonable to speculate that the initial binding of the metal ions to uncomplexed ds2 strands could induce larger structural perturbations, reflected in its Raman signature, than the insertion of a second Hg\(^{II}\) into the remaining T:T pair when \( R_{\text{Hg}} \) is increased.

To further demonstrate the reliability of the direct SERS analysis in differentiating and recognizing dsDNA complexes, partial least-squares discriminant analysis (PLS-DA) was performed based on the SERS spectra from ds2, ds2CP, and ds2Hg\(^{II}\). PLS-DA is a supervised classification method that is 473

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used to discriminate different groups. The ds2 was chosen as model dsDNA since it contains T:T mismatches to coordinate the mercuric ion and, in the same manner as ds1, forms covalent adducts with CP. From the established model, the three sample groups are distinguishable from each other with 100% sensitivity and specificity (Figure S10).

Genomic dsDNA. High-quality double-stranded DNA isolated from the thymus of calves (CTds) was used as a model genomic DNA for studying the interaction with CP and MB. Figure 5 shows the SERS spectra of CTds (7.8 μg/mL) and their complexes with CP and MB, as well as the corresponding digitally subtracted spectra. The characteristic spectral fingerprints of the CP adduct and MB intercalation are well highlighted in the difference spectra, in which the peak positions nicely match those observed for ds1. On the other hand, differences in relative band intensities can be ascribed to the different DNA features (composition, base sequence, and structural form). Similarly, the corresponding spectral ratios $I_{1588}/I_{1485}$ (Figure S8B,C, for CP adducts) and $I_{1624}/I_{1575}$ (Figure S8D,E, for MB complexes) show linear responses in the investigated ranges of nanomoles of MB per milligram of DNA (to top to bottom: 15.4, 5.1, 2.6, 1.3, and 0). (E) Ratiometric peak intensities $I_{1624}/I_{1575}$ vs MB/CTds ratio (nmol/mg). The concentration of CTds was kept constant at 7.8 μg/mL for the whole study, whereas CP and MB amounts were modified accordingly.

Adsorption of ds1 on the AgNP@Sp Model System. To gain a further understanding of the interaction between dsDNA and AgNP@Sp, we simulated the adhesion of ds1 onto a silver surface functionalized with spermine molecules in contact with electrolyte solution by means of molecular dynamics (MD) methods.

Starting from a common initial structure, five independent MD simulations (A1−A5) were carried out for a total sampling time of 50 ns each. The main goal of these calculations was to characterize possible binding modalities of ds1 on the AgNP@Sp model, and eventually to highlight important conformational changes on the oligonucleotide sequence upon binding.

For all the simulations, three structural regimes could be distinguished. These regimes were classified as (i) an initial recognition phase, where the oligonucleotide approaches the silver surface until the first contacts between phosphate groups and the positively charged nitrogen atoms of spermine are established; (ii) a subsequent relaxation phase, where the encounter complex evolves toward a more stable conformation; and finally (iii) an adhesion phase, where the DNA can be considered completely attached to the surface. The three phases are separated by the characteristic times $t_1$ and $t_2$, which are defined as the times required to form the first DNA−Sp contact and to reach a plateau in the DNA−Ag distance, respectively (see Table S1). In particular, a constant value in the DNA−Ag distance is achieved whenever the DNA sequence is completely lying down on the spermine-bound silver surface in an extended conformation (total adhesion). Hereafter, the general trends that emerge from simulations will be commented upon using results from simulation A1 as a prototypical case. A detailed description of differences among
the simulated systems can be found in the Supporting Information (Figures S11–S14).

**Recognition.** As reported in Figure 6A, at the beginning of the production runs ds1 was located about 2 nm from the closest spermine molecule (DNA–Ag distance ca. 3.2 nm). From this starting configuration, we observed a rather fast recognition process occurring on the order of 9–15 ns (see Table S1), meaning that the oligonucleotide sequence approached the positively charged silver surface at an average speed of more than 0.15 nm/ ns. During this recognition phase, ds1 experienced typical deviations from the ideal B structure (root-mean-squared displacement, RMSD, of the order of 0.3–0.4 nm) as well as a moderate bending of the double-strand axis (Figure 6B).

**Relaxation.** After the recognition, metastable complexes were observed to increase the number of favorable contacts with the help of spermine molecules that were freely diffusing on the top of the silver surface plane. As one might expect, both the duration and mechanism followed to achieve a stable structure were found to be strongly dependent on the geometry of the encounter complex reached at the end of the previous phase. In simulation A1, a significant increase in RMSD and in total axis bending was observed, reflecting the dramatic deformations experienced by the double strand in optimizing contacts. This behavior can also be appreciated by the local axis bending per base pair plotted against time (Figure 6C). As reported in Figure 6D, the relaxation phase in A1 was initially triggered by a spermine molecule binding to the minor groove of the duplex at the beginning of the sequence (1–3 bps in the S′–S′ strand). This first interaction was later reinforced by a second spermine molecule contacting the major groove oriented toward the Ag plane.

**Adhesion.** In order to grasp some insights on the equilibrium properties of the complexes, all simulations were additionally extended for tens of nanoseconds after the total DNA adhesion on the sliver surface. As it can be noticed from the RMSD and total axis bending shown in Figure 6B, as well as the average values reported in Table S2, adhesion on the surface resulted in a stiffening of the DNA structure. This behavior was reasonably expected as a consequence of the reduction in the DNA degrees of freedom due to tight interaction with a solid phase. In Figure S15, the occupancy of spermine nitrogen atoms in the proximity of ds1 calculated during the adhesion phase and averaged over all the simulations is shown. Several local density maxima can be distinguished, especially in the proximity of the minor groove, even though the major groove was also contacted. In the same figure, details on representative DNA–Sp binding modalities, as observed in system A1, are also reported. As it can be seen, thanks to their flexibility, spermine molecules are able to optimally fit into the minor groove of the DNA. At the same time, the extended conformation of the molecule is long enough to bridge the phosphate groups of the major groove.

The outcomes of the MD modeling of the DNA interaction with the AgNP@Sp are fully consistent with the experimental results. In fact, the final adhesion of the DNA structure in an extended conformation onto the silver surfaces is a key prerequisite for obtaining reproducible and intense SERS spectra which are truly representative of the nucleoside organization into a double helix.

**CONCLUSIONS**

In summary, we report a fast, high-throughput, flexible, low-cost method for the characterization and quantitative recognition of DNA interactions with exogenous agents based on label-free SERS spectroscopy. High-quality SERS spectra of unmodified duplexes and their complexes with a chemo-therapeutic drug (cisplatin), a DNA intercalator (methylene blue), and a toxic metal ion (Hg(II)) were obtained with high reproducibility and are independent of the DNA length (short oligonucleotides or genomic DNA). Binding of DNA with the damaging agents is revealed by the unique and characteristic vibrational alterations of the SERS spectra. A detailed analysis of the vibrational pattern provides both rich structural information regarding the perturbation of the DNA structure and an efficient analytical tool for identification and quantitation of the relative extent of the complexation.

**ASSOCIATED CONTENT**

Supporting Information

Materials and methods; SERS background of AgNP@Sp and CP; SERS spectra of ds2; SERS of CTDs at different concentrations; DCS analysis of AgNP@Sp and their DNA-induced aggregates; SERS spectra of ds1CP mixtures at

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**Figure 6.** Time evolution of the ds1 adsorption on the AgNP@Sp model for system A1. (A) Distance calculated between the DNA and the silver surface (light orange line on the left y-axis), and between the DNA and the spermine molecules (dark orange line on the left y-axis), plotted against the simulation time. The total number of DNA–Sp contacts is also shown (green line on the right y-axis). Changes in global structural properties (RMSD, dark orange line on the left y-axis, and total helix bending, green line on the right y-axis) and local properties (helix axis per base pair) of ds1 are plotted against time in panels (B) and (C), respectively. (D) Configurations of the system at the characteristic times t1 and t2 (see Supporting Information for details). The arrow shows the first DNA–Sp contact formed during the simulation.
different molar ratios; SERS of MB and single-stranded DNA; SERS spectra of Pt and its complex with Hg; SERS spectra of ds1 in the presence of Hg; ratimotoc peak intensities vs R_Hg; and additional results from MD simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

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Supporting information:

Revealing DNA Interactions with Exogenous Agents by Surface-Enhanced Raman Scattering

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Materials and methods

Materials

All materials were of highest purity available and obtained from Sigma Aldrich (St. Louis, United States), unless stated otherwise. DNA oligonucleotides were purchased from Eurofins Genomics (Esbjerg, Denmark). The oligonucleotide base sequences are as follows: (ss1) CATCGCAGGTACCTGTAAGAG; (ss2) CATCGCAGGTTCTCTGTATGAG; and (ssc) GTAGCGTCCATGGACATTCTC. Stock solutions (0.4 mM) of each oligonucleotide were prepared dissolving them in Milli-Q water (18.2 MΩ·cm at 25 °C). Annealing of ss1 and ss2 with the complementary ssc was conducted by heating to 95 °C for 10 minutes equimolar solutions of oligonucleotides in PBS (0.3 M). This yielded the corresponding double-stranded DNA solutions ds1 and ds2 (final concentration 20 μM) which were stored at -20 °C until required. As a reference sample for the Hg²⁺ coordination study, a 20 base homopolymeric sequences pT was selected.

Deoxyribonucleic acid from calf thymus (Type XV, Activated, lyophilized powder) was purchased from Sigma Aldrich. Stock solution (320 μg/mL) was prepared in PBS 0.3 M and stored at -20 °C until required.

Preparation of Positively-Charged Silver Nanoparticles (AgNP@Sp)

Spermine coated-silver nanoparticles (AgNP@Sp) were prepared as previously reported.¹ ² Briefly, 20 μL of a 0.5 M AgNO₃ solution were added to 10 mL of Milli-Q water, followed by addition of 7 μL of a 0.1 M aqueous spermine tetrahydrochloride. Subsequently, under vigorous stirring, 250 μL of a freshly prepared NaBH₄ (0.01 M aqueous solution) were quickly added to the mixture under vigorous stirring. Finally, the solution was gently stirred for further 20 min. Colloids were left to age overnight and the visible sediment at the bottom of the vial was eliminated from the sample. AgNP@Sp were characterized by ζ-potential (ca. +38 mV), transmission electron microscopy (TEM) (average diameter
of ca. 29 nm), UV-visible spectroscopy (surface plasmon peak centered at 391 nm, [NP] ca. 0.3 nM) and differential centrifugal sedimentation (DCS) analysis. Nanoparticle concentration was calculated by Lambert-Beer’s law using the extinction coefficient for silver nanoparticles of 1.85x10^{10} M^{-1} cm^{-1}, derived from literature.\(^1,^4\) Glass vials used for the reaction and storage of the particles, were pre-coated with polyethylene imine (PEI, average Mw ca. 25000 by LS) by an overnight immersion into an aqueous 0.2% v/v PEI solution, followed by extensive rinsing with fresh Milli-Q water and N\(_2\) dried. PEI is a cationic polymer that is used to neutralize the negative charge of glass by creating a positively-charged surface that prevents the electrostatic deposition of the AgNP@Sp colloids and prolonging shelf-life of the colloidal dispersion.

**SERS Experiments**

For SERS studies, 2.5 μL of PBS (0.3 M) solutions of (i) short dsDNA ds1 and ds2 (4 μM) and its complexes; or (ii) genomic CTds (320 μg/mL) and its complexes were mixed with 100 μL of AgNP@Sp ([NP] ca. 0.3 nM). The final analyte concentrations in the samples were 1.3 μg/mL for short dsDNA, and 7.8 μg/mL for genomic CTds. After the addition of the DNA, the colloids were left to equilibrate for 2 h and redispersed by quick sonication before running the SERS measurements. SERS studies of short single-stranded DNAs (ssc and pT) were performed as for duplexes except for the final analyte concentration in the sample (0.5 μM, corresponding to ca. 3.15 μg/mL).

*Formation of DNA adducts by cisplatin.* A set of samples at different CP/ds1 molar ratio were prepared by mixing 80 μL of the 2 μM ds1 solution to 1 μL of fresh CP aqueous solutions at different concentrations. Similarly, 80 μL of the 320 μg/mL ctDNA solution were mixed with 1 μL of fresh CP aqueous solutions at different concentrations. The mixtures were left to stand overnight at 4 °C and then investigated by SERS.
Intercalation of the organic dye methylene blue into the duplex. A set of samples at different MB/ds1 molar ratio were prepared by mixing 80 μL of a 2 μM ds1 solution to 5 μL of MB ethanolic solutions at different concentrations. Similarly, 80 μL of the 320 μg/mL ctDNA solution were mixed with 5 μL of MB ethanolic solutions at different concentrations. The mixtures were left to stand overnight at 4 ºC and then investigated by SERS. The final ethanol content in the mixture was kept very low (water/ethanol 16:1 v/v), at concentrations where the DNA structural stability is not compromised.5 Moreover, we performed experimental controls verifying that the SERS spectrum of ds1 (in pure buffer solution) does not show any significant alteration when the DNA samples is dissolved into the aqueous/ethanol mixture (16:1 v/v).

DNA-metal ion coordination: formation of T-HgII-T base pairs. A set of samples at different HgII/ds2 molar ratio were prepared by mixing 80 μL of a 2 μM ds2 solution to 1 μL of fresh HgCl2 ethanolic solutions at different concentrations. The mixtures were left to stand overnight at 4 ºC and then investigated by SERS.

Equipment and Instrumental Settings

UV-vis spectra were recorded using a Thermo Scientific Evolution 201 UV-visible spectrophotometer. FEI Tecnai G2 20 Twin TEM operating at accelerating voltage of 200 kV was used for imaging. The ζ (Zeta) potential measurements were carried out using a Malvern 2000 Zetasizer, using the default method protocol and a minimum sample volume of 3 ml. Before each measurement a standard solution of -68.0 ± 6.8 mV was measured.
For DCS analysis of the nanoparticle aggregates a CPS disc centrifuge DC24000 (CPS Instruments Inc.) was used operating at rotating speed of 18000 rpm. A 8-24 w/w % sucrose gradient in Milli-Q water (18.2 MΩ·cm at 25 °C) was freshly prepared and filled successively in nine steps into the disc starting with the solution of highest density followed by addition of 0.5 mL dodecane that was injected to prevent changes in the concentration by evaporation of aqueous gradient. Calibration before each measurement was performed using polyvinylchloride (PVC) standard particles (0.476 μm, Analytik Ltd.). For analysis, 0.1 mL of the sample was injected into the disc followed by detection at of the light source detector. The CPS software using the Stoke’s law calculates diameters referring to the density of silver i.e. 10.49 gcm⁻³.

SERS experiments were conducted using a Renishaw InVia Reflex confocal microscope equipped with a high-resolution grating consisting of 1800 grooves/cm for visible wavelengths, additional band-pass filter optics, and a CCD camera. A 532 nm laser was focused onto the colloidal solution by a long-working distance objective (0.17 NA, working distance 30 mm) and the spectra were acquired at room temperature with an exposure time of 5×10 s and a laser power at the sample of 6.9 mW. All SERS spectra reported in the manuscript as well as the data plotted in Figure 2C, 3C, 4C, 5C and 5E were obtained by averaging the SERS responses of 5 different sample replications (N=5, two measurements for each replica) obtained by combining the same colloids (prepared the day before the measurement and left aging overnight) with the specific DNA solution. Baseline correction was applied to all spectra, unless stated otherwise. Importantly, in the case of difference SERS spectra, the baseline correction was performed after the digital subtraction between the original no baseline-corrected spectra to avoid any generation of spectral artefacts.

**Computational Methods**

**PLS-DA analysis.** All the selected SERS spectra were preprocessed using weighted least squares baseline correction, normalized using Multiplicative Scatter Correction and mean centered before
being imported for modeling. Preprocessed data were imported to the PLS tool box (Eigenvector Research, Inc.) in Matlab for PLS-DA modeling. Four latent numbers were chosen representing 98.51% of data.

*Model Systems.* The interaction between dsDNA and AgNP@Sp was simulated by employing a model system consisting of 21 base pair oligonucleotide and a silver surface adsorbed with spermine molecules in contact with electrolyte solution. The (111) plane of the face-centered cubic lattice was chosen as adsorbing interface of the metal, and it was aligned to the xy plane of the simulation cell. The surface was prepared by repeating in space a smaller patch obtained from the INTERFACE 1.0 force field pack. The final system consisted of six silver layers spanning a surface area of approximately 112 nm² for a total number of 9324 Ag atoms. Nine regularly spaced spermine molecules were placed in close contact with one side of the (111) silver plane in such a way to homogeneously cover the whole surface. This number of spermine molecules was chosen so as to obtain an average charge density of more than one ionized group per 3 nm², which is expected to be roughly consistent with a surface potential of about +40 mV in similar experimental conditions. A double-stranded B-DNA model with the 5’-CAT CGC AGG TAC CTG TAA GAG-3’ sequence (ds1) was built with the automated Nucleic Acid Builder language. The dsDNA model was arranged in the simulation cell so that the helix axis was parallel to the metal surface, and by keeping a minimum distance of about 2.3 nm between them. The whole system was then solvated with the *tleap* module of the AmberTools12 package, and the electroneutrality of the cell was ensured by randomly replacing water molecules with Na⁺ and Cl⁻ ions consistently with an ionic concentration of about 0.1 M. The final system size was ≈ 110,000 atoms (*Figure S10*).

*Force Field Parameters.* The Lennard-Jones parameters for Ag were those developed by Heinz and coworkers. Although electronic polarization effects are not explicitly taken into account, these parameters have proven to provide a good energetic description for the adsorption process of peptides.
and surfactants with metal surfaces. Since we were here interested in studying the dsDNA adsorption on the surface mediated by the positively charged spermine molecules, which is a process mostly driven by permanent electrostatics, the use additive potentials represents an optimal tradeoff between accuracy and speed of calculations. Moreover, contrarily to gold surfaces, to the best of our knowledge, no polarizable force fields for the interaction of silver with nucleotides are available yet. The parmBSC0 force field was used for the dsDNA model, whereas the spermine molecule in the tetra-protonated form was parameterized according to the General Amber Force Field procedure together with RESP charges. RESP charges were derived from the electrostatic potential calculated at the HF/6-31G(d)//HF/6-31G(d) level of theory with the Gaussian 03 package. The TIP3P model and the Joung and Cheatham parameters were used for water and ions, respectively.

Molecular Dynamics Simulations. Molecular dynamics (MD) simulations were run on a NVIDIA Tesla C2050 GPU architecture with Amber12. The pmemd.MPI module was used with the SPFP mixed precision model. Langevin dynamics was performed at the nominal temperature of 298 K using a frictional coefficient of 5 ps, whereas the Berendsen barostat under isotropic position scaling was employed in the equilibration stage at the target pressure of 1 bar and with a relaxation time of 2 ps. Bonds involving hydrogen atoms were restrained to their equilibrium geometry with the SHAKE algorithm. A short-range cutoff of 1.2 nm was used for non-bonded interactions, whereas the Particle Mesh Ewald method was employed for long-range electrostatics with a grid of approximately 0.1 nm in all directions, and a fourth order spline as interpolation scheme. The equations of motion were integrated using a time-step of 2 fs, and the neighbor list was updated every 10 integration steps. The system was first energy minimized with repeated cycles of steepest descent and conjugate gradient. Then, the simulation cell was heated up to 298 K during 300 ps of MD performed in the canonical ensemble, and further equilibrated for 2 ns in the isothermal-isobaric ensemble. During this
equilibration stage, the positional restraints initially applied to the atoms of the system were gradually reduced until complete release. To achieve a better statistics, five independent production runs of the duration of 50 ns each (referred to as A1, A2, A3, A4, and A5 simulations) were therefore performed in the canonical ensemble through random assignment of initial velocities.

**Structural Analysis.** Conformational changes in the dsDNA model upon adsorption on the AgNP@Sp were monitored by calculating the root mean squared displacement (RMSD) of the deoxyribose ring C1’ atoms over time, using the ideal B-DNA structure as reference. The analysis was performed with the ptraj module of Amber12. The helical properties of the double strand were determined with Curves+. The z-axis component of the distance between the DNA center of mass and the silver surface as well as and the minimum distance between oxygen atoms of the phosphate groups and spermine nitrogen atoms (labeled as DNA-Ag and DNA-Sp distance in Figure 7 and in the “Additional results from molecular dynamics simulations” section of the Supp. Inf.) were calculated with PLUMED-1.3. The total number of contacts between DNA and spermine molecules (DNA-Sp contacts) was monitored via Tcl scripting running within the VMD-1.9 visualization package, using a threshold of 0.3 nm in the distance between the oxygen atoms of the phosphate groups and the nitrogen atoms of spermine. VMD-1.9 was also used to compute the occupancy of spermine’s nitrogen atoms in the vicinity of the DNA after the surface adhesion.
**Figure S1.** Background SERS spectrum of AgNP@Sp (black curve) aggregated with 0.1 M MgSO$_4$. MgSO$_4$ is a “passive” electrolyte that induces nanoparticle aggregation by simply increasing the ionic concentration without firmly adsorbing onto the silver surface. SERS spectra AgNP@Sp colloids in the presence of HgCl$_2$ (final concentration 5 µM) and CP (final concentration 5 µM and 50 µM). The colloidal mixtures were aggregated with MgSO$_4$ 0.1 M before the SERS measurement. The final concentration of Hg$^{II}$ in the colloids corresponds to the amount contained in the hypothetical ds2Hg$^{II}$ samples for $R_{Hg}=100$, whereas in the case of CP, the final concentrations of the chemotherapeutic drug, 5 µM and 50 µM, correspond to the hypothetical values $R_{CP}=100$ and 1000, respectively, for the ds1CP SERS study. In the case of Hg$^{II}$, we do not observe any new contribution in the SERS spectra of AgNP@Sp colloids, whereas a new intense band at 541 cm$^{-1}$ emerges for CP, which can be ascribed to the Pt-NH$_3$ stretching of CP ligands, as well as a weaker feature at 835 cm$^{-1}$.
Figure S2. Original no-baselined SERS spectra of 9 different repetitions of AgNP@Sp + ds2 (1.3 μg/mL). The repetitions were performed as follows: 100 μL of AgNP@Sp from the same colloidal batch were placed in 8 different vials, then mixed with the same volume of a ds2 buffered solution and finally investigated by SERS. As can be seen in the figure, only minimal fluctuations of the background and overall SERS intensity are observed whereas the spectral profile remains unperturbed, showing a consistent sample-to-sample reproducibility. Importantly, our study relies on the analysis of (the changes in) the SERS spectral profiles and not on the absolute SERS intensity. Therefore, minor changes in the background and overall SERS intensity do not affect the final outcome.
Figure S3. Non-baselined SERS spectra of CTds at different concentrations ([NP] ca. 0.3 nM). Generation of intense SERS spectra is associated with both the adhesion of a sufficient amount of analyte onto the metal surface and the extended DNA-induced nanoparticle aggregation (i.e. formation of interparticle hot-spots). The first condition is not satisfied at low DNA/NP ratios (CTds concentration approximately below 2.0 µg/mL) whereas the second condition is not fulfilled at high CTds/NP ratios (CTds concentration approximately above 30 µg/mL) when the excess of analyte saturates the silver surface limiting the formation of clusters and short interparticle junctions.
**Figure S4.** DCS measurement of the weight distributions (linear scale) of AgNP@Sp nanoparticles; and ds1- and dsCT-mediated AgNP@Sp aggregates analyzed *via* DCS at 18000 rpm. The final concentration of DNA was 0.1 µM, corresponding to 1.3 µg/mL, for ds1, and 7.8 µg/mL for CTds. The AgNP@Sp concentration was kept constant to ca. 0.3 nM.
**Figure S5.** Original non-baselined SERS spectra of ds1 and ds1CP mixtures at different $R_{CP}$ molar ratios. The final ds1 concentration was kept fixed through the entire study at 1.3 µg/mL whereas the CP concentration was modified accordingly. No significant changes of the overall SERS intensity for DNA/CP complex were observed in the investigated $R_{CP}=0$-50 range.
**Figure S6.** Interaction of methylene blue (MB) with single-stranded DNA (ssc). SERS spectra of ssc+MB complex (1:1 molar ratio), ssc and MB. The final ssDNA concentration in the sample was kept fixed at $5 \times 10^{-7}$ M (corresponding to 3.15 µg/mL). The difference spectrum sscMB - ssc reveals intense features mainly ascribed to MB which largely matches those of the SERS spectrum of the dye molecule directly adsorbed on AgNP@Sp.
Figure S7. SERS spectra of pT (20 thymine bases) and the equimolar pTHg\textsuperscript{II} mixture (0.5 µM, corresponding to 3.1 µg/mL for pT). The corresponding difference spectrum (pTHg\textsuperscript{II} - pT) is included in the figure.
**Figure S8.** SERS spectra of ds1 solution and the equimolar ds1Hg$_{II}$ mixture (ds1 concentration 1.3 µg/mL). The corresponding difference spectrum (ds1Hg$_{II}$-ds1) does not reveal any spectral changes of the duplex upon addition of the metal ions, providing a further evidence of the specific and selective Hg$_{II}$ interaction with thymine residues in T-T mismatches.
**Figure S9.** Ratiometric peak intensities $I_{780}/I_{795}$ vs. $R_{Hg}$ molar ratio (linear scale). The values correspond to the data reported in Figure 4C in logarithmic scale.
Figure S10. Partial least squares discriminant analysis (PLS-DA) of the SERS spectra from ds2 and their complexes ds2CP and ds2Hg\textsuperscript{II}. (A) Latent number score plot of the obtained SERS spectra with 98% confidence ellipses. (B) VIP score map of the obtained SERS spectra. The major differences are highlighted and ranked by the intensities. The reported spectra are the average spectra of more than 30 measurements (6 independent repeats) per sample.
Additional results from molecular dynamics simulations

In three out of five simulations (A2, A4, and A5) the first contacts between DNA and the spermine molecules were established through the phosphate groups located in the middle of the duplex (see Figure S11D, 13D, and 14D, respectively). By contrast, the end tail of the oligonucleotide sequence was found to be directly involved in recognition in A1, whereas A3 was the only case were the silver surface was contacted by DNA before the spermine molecules (Figure S12D).

During relaxation, A1, A2, and A3 displayed a significant increase in RMSD and/or in total axis bending (Figure 13B, S11B and S12B, respectively). On the contrary, the relaxation in A4 and A5 appears to be occurred in a much smoother way, and this is especially true for the latter simulation which lasted only about 6 ns (Figure S13A and S14A). As reported in the main text, the relaxation in A1 was triggered by a spermine molecule binding to the double strand tail, in correspondence of the minor groove. However, this picture of binding was found to be reversed in A3 – A5 where the relaxation was triggered by spermine binding to the major groove and was only assisted by minor groove binding. As a matter of fact, A2 was the only simulation were the DNA relaxation occurred without involvement of the minor groove.

In the adhesion phase, we observed for all the systems a further increase in the number of DNA-Sp contacts, reaching a maximum of 13 interactions in A2, for a total number of 6 bound spermine molecules (Figure S11A). As mentioned in the main text, the adhesion on the silver surface resulted in a stiffening of the DNA structure in A1, A2, and, though less noticeable, in A5 (Figure 13B, S11B, and S14B). In system A3 and A4, however, this was not apparently the case. By looking at the total axis bending against time for A3 (Figure S12B) it is possible to notice a significant bending of the double strand starting from the beginning of the relaxation phase. The local axis bending is even more informative in that it shows that the curvature was mostly concentrated at the first half of the sequence.
(bps 1 – 12, Figure S12C). A visual inspection of the trajectories revealed that this deformation could be imputed to a spermine molecule bridging the corresponding major groove and causing the conformational alteration that lasted until about 42 ns of simulations. Contrarily to the previous case, in A4 the above reported stiffening effect was also present, but it is partially hidden by the remarkable stability showed by the DNA in the preceding phases. Moreover, approximately in correspondence of 35 ns of simulation, the nucleobases of the first bp of the sequence started to interact directly with the Ag surface causing a large deformation in the DNA structure, as it is reflected by the remarkable local axis bending in Figure S13C. Even though the possibility of this interaction cannot be a priori ruled out, further investigations would be necessary to ascertain whether no artifacts related to the simplified model used to treat the silver surface were incidentally responsible for such an event.

Table S1. Characteristic times used to classify the three structural regimes observed for ds1 upon adsorption on the AgNP@Sp model.

<table>
<thead>
<tr>
<th>Simulation</th>
<th>$t_1$ (ns)</th>
<th>$t_2$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>9.00</td>
<td>23.37</td>
</tr>
<tr>
<td>A2</td>
<td>14.67</td>
<td>26.81</td>
</tr>
<tr>
<td>A3</td>
<td>9.42</td>
<td>17.83</td>
</tr>
<tr>
<td>A4</td>
<td>9.99</td>
<td>18.61</td>
</tr>
<tr>
<td>A5</td>
<td>11.99</td>
<td>18.04</td>
</tr>
</tbody>
</table>
Table S2. Averages and standard deviations for the most relevant structural properties observed for ds1 upon adsorption on the AgNP@Sp model.

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Recognition</th>
<th>Relaxation</th>
<th>Adhesion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RMSD (nm)</td>
<td>Total axis bending (deg)</td>
<td>RMSD (nm)</td>
</tr>
<tr>
<td>A1</td>
<td>0.30 ± 0.07</td>
<td>20.1 ± 10.4</td>
<td>0.32 ± 0.06</td>
</tr>
<tr>
<td>A2</td>
<td>0.35 ± 0.07</td>
<td>22.2 ± 10.1</td>
<td>0.46 ± 0.07</td>
</tr>
<tr>
<td>A3</td>
<td>0.31 ± 0.05</td>
<td>20.7 ± 9.6</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>A4</td>
<td>0.34 ± 0.08</td>
<td>18.4 ± 9.3</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td>A5</td>
<td>0.42 ± 0.08</td>
<td>26.8 ± 12.8</td>
<td>0.44 ± 0.07</td>
</tr>
</tbody>
</table>
Figure S11. Time evolution of the ds1 adsorption on the AgNP@Sp model for system A2. (A) Distance calculated between the DNA and the silver surface (light orange line on the left y-axis), and between the DNA and the spermine molecules (dark orange line on the left y-axis), plotted against the simulation time. The total number of DNA-Sp contacts are also shown (green line on the right y-axis). Changes in global structural properties (RMSD, dark orange line on the left y-axis, and total helix bending, green line on the right y-axis) and local properties (helix axis per base pair) of ds1 are plotted against time in panels (B) and (C), respectively. (D) Configurations of the system at the characteristic times $t_1$ and $t_2$ (see Materials and Methods for details).
Figure S12. Time evolution of the ds1 adsorption on the AgNP@Sp model for system A3. (A) Distance calculated between the DNA and the silver surface (light orange line on the left y-axis), and between the DNA and the spermine molecules (dark orange line on the left y-axis), plotted against the simulation time. The total number of DNA-Sp contacts are also shown (green line on the right y-axis). Changes in global structural properties (RMSD, dark orange line on the left y-axis, and total helix bending, green line on the right y-axis) and local properties (helix axis per base pair) of ds1 are plotted against time in panels (B) and (C), respectively. (D) Configurations of the system at the characteristic times $t_1$ and $t_2$ (see Materials and Methods for details).
Figure S13. Time evolution of the ds1 adsorption on the AgNP@Sp model for system A4. (A) Distance calculated between the DNA and the silver surface (light orange line on the left y-axis), and between the DNA and the spermine molecules (dark orange line on the left y-axis), plotted against the simulation time. The total number of DNA-Sp contacts are also shown (green line on the right y-axis). Changes in global structural properties (RMSD, dark orange line on the left y-axis, and total helix bending, green line on the right y-axis) and local properties (helix axis per base pair) of ds1 are plotted against time in panels (B) and (C), respectively. (D) Configurations of the system at the characteristic times $t_1$ and $t_2$ (see Materials and Methods for details).
Figure S14. Time evolution of the ds1 adsorption on the AgNP@Sp model for system A5. (A) Distance calculated between the DNA and the silver surface (light orange line on the left y-axis), and between the DNA and the spermine molecules (dark orange line on the left y-axis), plotted against the simulation time. The total number of DNA-Sp contacts are also shown (green line on the right y-axis). Changes in global structural properties (RMSD, dark orange line on the left y-axis, and total helix bending, green line on the right y-axis) and local properties (helix axis per base pair) of ds1 are plotted against time in panels (B) and (C), respectively. (D) Configurations of the system at the characteristic times \( t_1 \) and \( t_2 \) (see Materials and Methods for details).
Figure S15. Pictorial representation of the occupancy calculated for the positively charged nitrogen atom of spermine around ds1. The density map is shown in transparent, and it is superposed to a representative configuration extracted from the A1 simulation. For clarity, only the interacting spermine molecules and the duplex are explicitly shown.
Figure S16. Initial configuration of the simulation box. Only part of the total number of water molecules is shown.
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