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Effects of drugs covalent binding on DNA: joint use of microRaman spectroscopy and HRTEM imaging☆

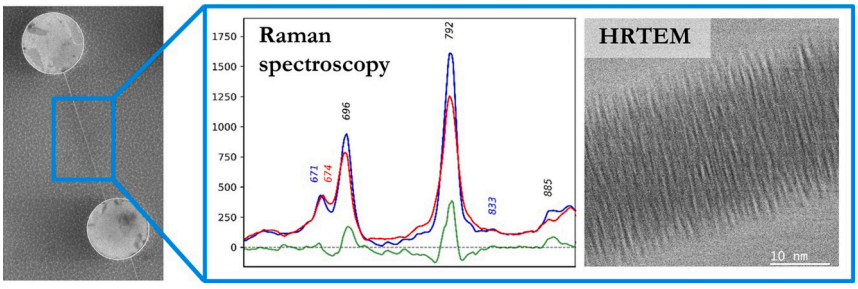
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HIGHLIGHTS

- Drugs bind to DNA to induce structural and chemical changes.
- Superhydrophobic devices enable background-, label-free study of DNA-drug adducts.
- Physiological and altered DNA state are identified by fine Raman spectra variations.
- HRTEM direct imaging confirms DNA local denaturation upon drug exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

The detailed understanding of drugs interaction with cells biomolecules is fundamental to evaluate the most efficient drug dosage. In this work we provide details on the structural modification occurring to DNA upon cross-links formation with metal ions after the administration of chemotherapeutic compounds. We used nanometric filaments of suspended DNA on superhydrophobic-based devices (SHS) for an accurate analysis by microRaman spectroscopy and high-resolution transmission electron microscopy (HRTEM) to study the interaction of cisplatin with the double helix. Our data show a conformational transition of the nucleic acids upon drugs administration, relying on Raman shift and intensity variations to features such as backbone vibration (792, 834 cm^{-1}), guanine ring breathing (~670 cm^{-1}), stretching modes of the adenine ring (~1300 cm^{-1}, 1338 cm^{-1}), unpaired AT bases (1178 cm^{-1} and 1204 cm^{-1}) and deoxyribosyl CH stretching vibrations (range 2800–3000 cm^{-1}). The conformational transitions towards a loosen DNA form and the integration of the drug into the double helix structures has been further confirmed by HRTEM, describing local helix denaturation. We demonstrated that the proposed methodology can be used to distinguish treated from pristine DNA by their Raman spectra, confirmed by the structural insights provided by direct imaging.

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1. Introduction

Cisplatin [cis-diammine-dichloroplatinum(II)] is the first metal-based anticancer drug approved by the Food and Drug Administration (FDA) in 1978 [1]. Since then, it has been used in the treatment of several types of cancers such as testicular cancer, ovarian cancer, lung cancer, and childhood brain tumors among others [2]. Cisplatin becomes active towards nucleic acids only upon the uptake and internalization in the cell and the anticancer effect is actuated via the formation of adducts with double-strand DNA in the nucleus [2]. Due to the lower chlorine concentration, cisplatin chlorine moieties can be released in favor of the complexation with up to two molecules of water [3]. Activated cisplatin then forms three main types of structures with DNA in a process named 'platination': mono adducts, intrastrand crosslinks and interstrand crosslinks [4]. Monoadduct formation is mainly transient as it is further converted into intra- and inter-strand crosslinks. The favored platination event allows the formation of intrastrand crosslinks in the 90 % of the cases and a cisplatin molecule is covalently bound to two nucleotides on the same strand [5]. In intrastrand crosslinking, cisplatin interacts with the nucleophilic residues of the bases, more precisely with the N7 atom of neighboring purine residues (1,2-GpG and 1,2-⟩GpA) [6]. This alteration promotes the anticancer effects of the drug [7] by triggering cell death [8,9]. For these reasons, the detailed understanding of the structural modifications induced by the interaction with its biological targets, which is at the basis of drug efficiency [10], resistance [11] and side effects, is crucial for the development of a personalized therapy and to determine the molecular processes behind the action of drugs.

Several studies focus on the relation between the structure of the complex drug-DNA and the cisplatin-nucleotide ratio and were carried out with well-established structural and spectroscopic techniques [12–14]. Conventional techniques such as X-ray diffraction [15–17], and Nuclear Magnetic Resonance [5,18,19], focused on the study of oligonucleotides and were of fundamental importance in resolving the structure of cisplatin-DNA adducts and in the assessment of the kinetics of the platination process. Crystallographic techniques have confirmed the binding geometry of cisplatin to the N7 position of guanine, as well as the intermolecular interactions between the ammine groups of cisplatin and the neighboring phosphate oxygen atoms [15]. These studies also revealed the significant alteration of the DNA double helix upon drug binding [17]. Notably, discrepancies were observed between structural data obtained from different techniques [12]. While NMR spectroscopy indicated severe distortions of the helical axis and an overall strained system, it consistently identifies the DNA as adopting a canonical B-form conformation; on the other hand, X-ray crystallography revealed the presence of both A- and B-form features within the same structure [17]. This divergence is generally attributed to crystal packing effects, which may stabilize high-energy conformational states that are unlikely to be populated under physiological conditions [12].

Raman spectroscopy studies on DNA identified several characteristic marker bands associated with different conformational states, as well as vibrational modes arising from the various nucleobases and organic moieties of specific nucleotides and their interactions [20–22]. In the specific case of cisplatin–DNA interactions, spontaneous Raman spectroscopy has revealed structural alterations in the DNA, primarily associated with a partial transition from B-form to A-form. Raman spectral signatures indicative of cisplatin binding at the N7 position of guanine have been observed, along with features consistent with DNA melting and pre-melting transitions [13,23–25].

DNA-ligand interactions were studies also by Surface-Enhanced Raman Spectroscopy (SERS), which is a highly sensitivity technique due to the plasmonic enhancement allowed by metallic nanostructures [26,27]. However, it is important to note that for extended molecules such as DNA, the SERS signal strongly depends on the fraction of the molecule in direct contact with the plasmonic substrate. As a result, significant spectral variability can arise between different measurements [28].

A consistent relation between sample preparation and spectroscopic signal has been also described [29]. It has to be noted that these investigations are based on the use of short-chained oligonucleotides, in the range of hundreds of base pairs, and therefore composed by a limited number of bases, which are not representative of the information dispersed through a cellular native DNA molecule [5,13–15,18,19]. Spectroscopic techniques, in particular Raman spectroscopy, have extended the analytical capabilities to longer DNA molecules (up to 2000–4000 bp) giving structural and chemical information about the variety of structure formed as well as their dynamics [30–33]. Nevertheless, the time-consuming preparation procedures needed to obtain the amount and the purity of material for adduct analysis is a limiting factor for their routinary application in the detection and description of structural alterations. Also, the type of preparation procedure affects the sample response itself. For instance, several spectroscopic studies used macroscopic samples such as crystals, fibers, hydrogels and films and therefore with sizes in the range of approximately 100–150 μm for fibers diameters to the millimetric sizes of crystals [20,21,34–37]. In these microstructures, the residual of hydration layers and buffer solutions remains an uncontrolled parameter, affecting the final spectroscopic response. Measurements of DNA in solution are often performed at high concentration (up to 25 mg/ml). Such solution conditions can contribute to the aggregation of the biomolecules and can contribute to undesired interactions between different helices [34]. Several differences are introduced by the presence of the background caused by the solvent and the physical state of the DNA sample. Raman studies on DNA are frequently performed in highly hydrated states, but several work describe Raman Spectroscopy on solid samples [38–41]. All these parameters impact the spectral signature of the DNA sample, creating significant variability in the spectral signatures reported in previous literature. Our preparation method prevents all these uncontrollable environmental spectral and structural results.

Due to these limitations, there is an urgent need to develop new methodologies for evaluating the structural effects of a therapeutic dose of cisplatin on nucleic acids from patients. Nowadays, the advances in micro- and nanofabrication as well as the improved sensitivity of photodetectors open a new frontier of investigations on nanoscopic 1-D samples, extending, in the field of structural biology, the investigation to samples closer to their native dimension, as electrospun DNA fibers of diameters of an average diameter as low as 35 nm, reported by Maleckis and Denis [42]. In this context, super-hydrophobic substrates (SHS) can be employed to establish an innovative sample preparation procedure, efficient in terms of sample concentration, down to 1 fM, purity and of a reduced time for sample preparation [43]. In the case of DNA, micropatterned substrates are able to concentrate the sample in the form of nanometric filaments, suspended and free standing on the patterned surface [44]. Indeed, during the drying process, non-interacting compounds are removed from the DNA filaments [45]. This method allows the retention of the structural, spectral, and physical features of the native biomolecule, while avoiding contamination with the buffering solution, as proved by previous studies [44–46]. Also, the suspended filaments obtained in the dehydration process have diameters ranging from 8 to 150 nm [47] while their length is of approximately 12 μm, consistent with the 50 kbases sequence of the DNA strand. The nanoscale dimensionality allows studying a reduced number of molecules, thus avoiding the contribution of inter-helical interactions typically occurring in crystal samples and preventing the formation of undesired non-physiological structures [34]. Filaments formation on SHS allows controlling their dimension and orientation. Due to the reduced dimensions of the suspended filament, the interaction of few molecules at a time can be investigated by multiple techniques, such as Laser Doppler Vibrometry [46,47], Atomic Force Microscopy [48], Electron Microscopy [49], and microRaman spectroscopy [50].

In this work, filaments entirely made of DNA and ligands were investigated by microRaman spectral analysis, which is a sensitive technique able to provide detailed information on the chemical and

structural alteration of long DNA molecules in the range of 50 kilobases [51] upon environmental stress (i.e. covalent binding of drugs) at sub molecular level. To this end, we combined superhydrophobic substrates, to suspend biomolecules on a background free platform and confocal microRaman spectroscopy to achieve local structural information on the interaction between cisplatin and double strand nucleic acids, at low DNA concentrations (~30 nM) and by using long DNA molecules (~50 k bases). To further confirm and verify the formation of cisplatin-DNA crosslinks, direct imaging by HRTEM was performed on the suspended DNA samples after drug administration.

2. Experimental

2.1. Super-hydrophobic surfaces fabrication

Super-hydrophobic samples of micropillar arrays were obtained following the procedure reported in previous works [52]. Briefly, Silicon ⟨100⟩ wafers were used as a substrate [53] and a circular pattern of disks was realized by optical lithography within a layer of positive photoresist, while metals were deposited by a sputter coating. The metals deposited were, in this order: a 10 nm layer of Ti to promote adhesion of gold on Si, followed by a 50 nm Au layer, which is protected by 50 nm of Cr. Pillars of an approximate height of 10 μm were obtained by etching in a DRIE system, then the Cr layer was removed with a selective wet etching. The sample was first covered with a 1-nm-thick layer of Al_2O_3, deposited by means of atomic layer deposition, and then functionalized with perfluorodecyltrichlorosilane (FDTS) in a Molecular Vapor Deposition System to enhance the super-hydrophobic features of the device. Substrates for HRTEM were fabricated similarly: the holes and the pillars were defined by two different lithographic steps and subsequently etched with two different Deep reactive ion etching processes [54].

2.2. DNA solution preparation and deposition

λ-DNA (48.5 kbp, New England Biolabs, Ipswich, MA, USA) was preheated 10 min at 65 °C before the incubation with a saturating amount of CisPt (Alfa Aesar, Karlsruhe, Germany), as previously assessed by the titration curve reported in [47]. The samples were incubated for 72 h at 37 °C [47] in aqueous solution. CisPt-DNA complex was diluted in saline buffer solution (6.5 mM NaCl, 10 mM Tris HCl, pH 9.3), reaching the final concentration of 1 ng/μl before use [49]. A 5 μl droplet of the final solution was then pipetted on the SHS and placed on a hot plate to keep a ΔT of 5 °C between the sample and the environment [55,56]. The same volume of the sample has been drop casted on CaF_2 windows as negative control and for preliminary screening. The same samples were analyzed by Circular dichroism with a Jasco J-815 photometer. CD spectra were obtained by working at a scanning speed of 100 nm/min, a DIT of 4 s, a bandwidth of 1 nm and by accumulating 10 spectra.

2.3. Characterization of suspended samples

2.3.1. Electron microscopy

The samples were imaged by Quanta 200 SEM (ThermoFisher Scientific, Waltham, US), working at an acceleration voltage of 3 kV and 25pA (substrates without holes) or 5 kV and at 43 pA of current (substrates with holes). HRTEM imaging was performed by using an aberration-corrected X-FEG Titan 60–300 TEM (ThermoFisher Scientific, Waltham, US) working at 80 keV and a Gatan Tridiem 865 image filter (GIF). The typical electron dose setting used for HRTEM imaging was about 10–20 electrons $Å^{-2}$/s, and the exposure time of the CCD camera of 0.3–1 s [57]. The obtained HRTEM images were then analyzed with Gatan software. The interbases distances were measured by generating the image FFT followed by the application of a spot mask for each of the four features visible in the FFT and the generation of the Inverse FFT (Fig. 2).

2.3.2. Raman spectroscopy

Raman spectroscopy characterization has been performed by a WITec confocal Raman system (α-Raman WITec GmbH, Ulm, Germany), working with a 100× Zeiss objective, with a grating of 600 g/mm, and equipped with a 100 μm core fiber and a Newtown CCD (Oxford Instruments, Abingdon, United Kingdom) thermoelectric cooled at −80 °C. A solid-state laser, 532 nm was used as the excitation line, working at a laser power of ~4 mW. Each measure was acquired with Control Five software (WiTec, GmbH, Ulm, Germany). As negative control, working buffer solutions were drop casted onto CaF_2 windows and the spectra acquired after the complete dehydration of the droplet. The samples analyzed were (i) cisplatin solution and (ii) cisplatin solution in working buffer on CaF_2 windows, (iii) suspended cisplatin-DNA adducts and (iv) suspended pristine DNA. The spectra of the suspended materials were obtained by accumulating 150 spectra, each with an exposure time of 3 s. Each spectra presented is obtained by the average result of at least 15 spectra acquired over 10 different suspended filaments. Spectra analysis has been carried out as previously reported [45]. The averaged spectra Briefly, Raman wavenumbers were individually re-centered by a Lorentzian fit on a reference peak (521 cm^{-1} for Silicon and 321 cm^{-1} for CaF_2) and subtracted by the background in the spectral region 200–3900 cm^{-1} [58]; the Gaussian broad peaks ascribed to water residuals in the region 2600–3800 cm^{-1} have been excluded from the background calculation. The spectral contribution of each peak emerging from the noise level was defined by fitting a set of Lorentzian curves in the background-subtracted data, while peaks with amplitude comparable with the noise level and with full width at half maximum (FWHM) < 2 cm^{-1} were not considered for further analysis. Large bands were fitted as a group of overlapping peaks. The number of peaks in each band was defined in order to maximize the R^2 adjusted parameter for each fit. For the evaluation of CisPt linkage to the DNA bases, spectra were normalized after background removal to the intensity of the peak centered at 1610 cm^{-1} while the suspended samples were normalized to the intensity of the peak falling in the range 1460–1470 cm^{-1} [59]. The region 900–1020 cm^{-1} has been neglected in data analysis to avoid misleading assignments due to the contribution of the Silicon secondary peak.

3. Results and discussion

The discussion of the results in this section is related firstly to a preliminary verification of the cisplatin binding to the DNA double helix through Circular Dichroism (CD). The CD spectra of pristine and platinated DNA are reported in Fig. S1 (Supporting Information). The administration of cisplatin at saturating conditions [47] slightly affects the CD spectra of pristine DNA and the variations observed are strongly dependent on the adducts formation. We should point out that in this work we used concentration at the limit of the detection of the CD technique to make them complementary with spectroscopic and HRTEM data which on the other hand show remarkable variations, demonstrating the enhanced capability of our approach. DNA-Cisplatin CD spectra show an increase in dichroic signal of the positive band located around 273 nm [60] and a blue shift; the dichroic signal of the negative band around 245 nm is slightly reduced while the positive band around 220 nm shows a more appreciable decrease in dichroic signal (Supplementary Fig. 1). Therefore, despite the slight differences between the CD spectra they are in agreement with previously published literature assessing that those changes are markers of a conformational transition between A and B forms [61].

The bundle suspension over SHS has been verified through SEM while the structural effects of the cisplatin-DNA adduct formation has been detailed by HRTEM direct imaging (Section 3.1). Finally, the analysis of Raman spectra (Section 3.2) allowed obtaining structural and chemical information on the modification caused by the formation of the

covalent bond between the chemotherapy intercalant and the DNA.

3.1. Filaments suspension and adducts validation by electron microscopy

The assessment of biomolecules suspension on SHS and the integration of the cisplatin molecule into the double helix has been preliminary provided through scanning electron microscopy (SEM) and HRTEM, respectively.

The dehydration of the droplet deposited over the SHS allows the suspension of free-standing cisplatin-DNA fibers. The filaments are autonomously oriented (Fig. 1) and intrinsically ordered, as assessed by previously published experimental evidence [54] and in silico simulations [47,49,52,62]. A droplet residual of approximately 450 μm of non-suspended materials and buffering agents is visible in Fig. 1 panel a, and is surrounded by cisplatin-DNA fibers of diameters ranging from 70 nm down to a few nm while moving towards the most peripherical area of the device. They are visible as fainted lines and highlighted by arrows in Fig. 1 panels c,d. Additional SEM images are reported in Supplementary Fig. 2.

A filament of approximately 20 nm has been directly imaged through HRTEM revealing periodic arrangements related to interbases distances (Fig. 2). To identify and measure the spacing between bases from HRTEM images (Fig. 2, panel a top image), Discrete Fourier Transform (DFT) was applied (Fig. 2, bottom image of panel a), giving four main spots. An image representing the localization of each set of spatial frequencies is obtained through the inverse discrete Fourier transform (Fig. S3, Supporting Information) after proper masking, and used to extract the spatial frequency information from the acquired image. The resulting intensity profiles are reported in Fig. 2, panel b, and describe the periodic features of the image in the direct space. The Inverse DFT shows a path overlapping the HRTEM direct image and clearly identifies a spatial distribution of different interbases distance. The line profiling of the Inverse FFT obtained is reported in Supplementary Fig. 3. In the suspended sample it is possible to distinguish between four different periodicities, ascribed to three main DNA structural conditions. Firstly, an interbase distance of 5.6 Å is related to DNA denaturation caused by the interaction with cisplatin (as previously reported in [47]), a distance of 3.3 Å corresponds to the DNA double helix in the hydrated form while the distances of 2.8 and 2.2 Å are related to DNA in the most dehydrated form [37,49,63,64]. The DNA filament is composed of concentric layers of aligned DNA double helices which retain an inter-helical hydration layer of water for structural stability, justifying the presence of features related to DNA hydrated B-form [62] while the outer shell retain less water molecules. The bundle conformation composed of multiple aligned DNA double helices, allows the concurrent presence of different interbases distances in the same area.

3.2. Raman spectroscopy: Characterization of the cisplatin-DNA interaction

To assess the cisplatin fingerprint, the spectral analysis of the chemotherapeutic solution was evaluated on CaF_2 windows. The spectra study reveals the presence of two main peaks at 1610 cm^{-1} and 1730 cm^{-1} (Fig. 3, panel b) assigned to cisplatin NH_3 in plane deformation [65] and its presence is the spctral confirmation of the drug integration into the double helix. The same peaks are detected in the working buffer added of cisplatin, along with Tris-HCl characteristic main bands in the spectral region 600–1800 cm^{-1} at 760, 1064, and 1467 cm^{-1} [66]. In

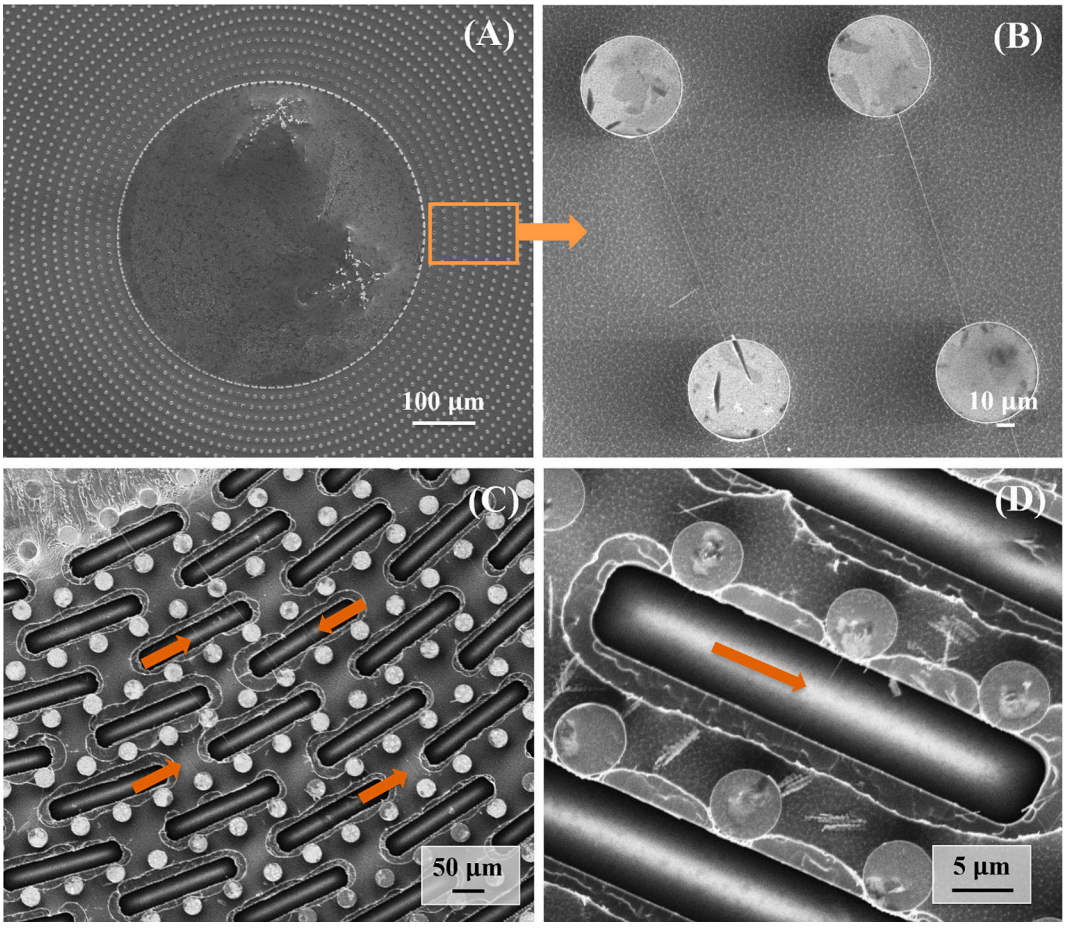


Fig. 1. SEM micrographs of circularly patterned SHS; a) low magnification picture of the drying residual b) DNA bundles suspended between two couples of pillars; c, d) DNA bundles suspended on top of the holes, which allow TEM imaging. Arrows indicate the presence of suspended DNA bundles on top of the holes.

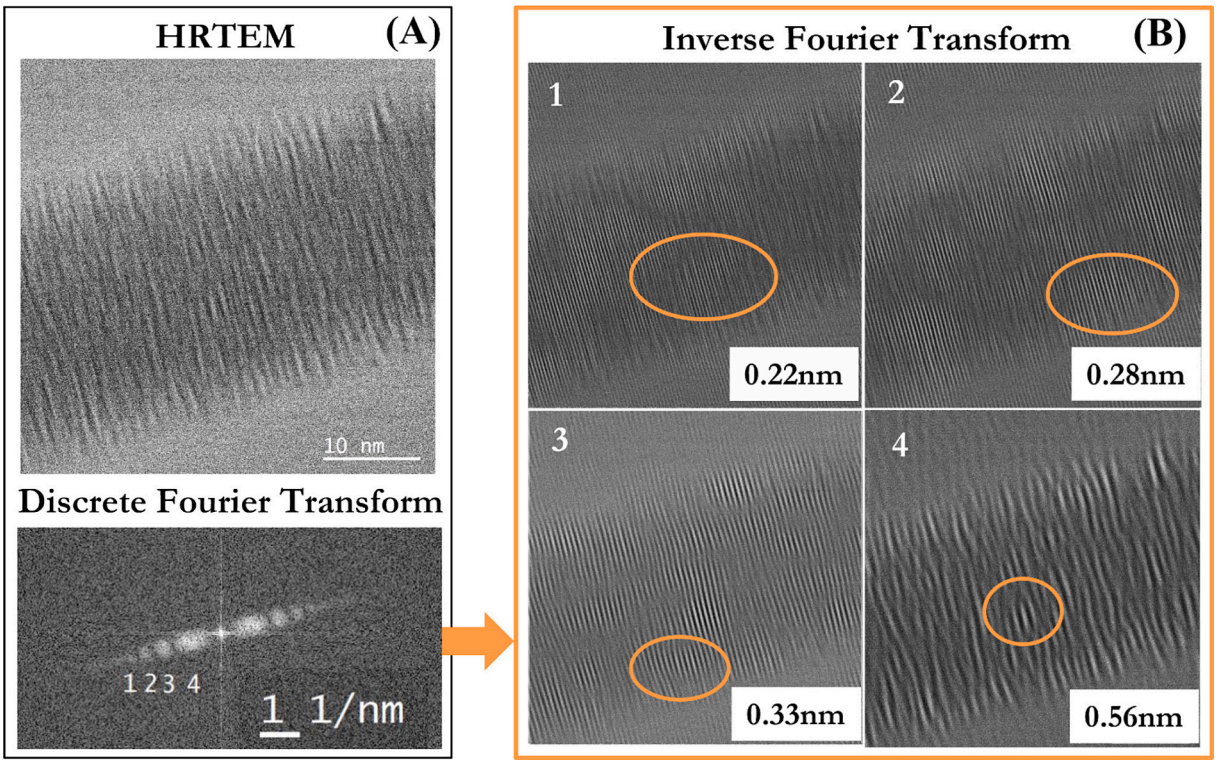


Fig. 2. (a) HRTEM of a 20 nm bundle of a cisplatin-DNA and the related Discrete Fourier Transform. (b) The Inverse Fourier Transform obtained by the analysis of the cisplatin-DNA adduct of the HRTEM image describes the simultaneous presence of different DNA interbase distances, related to less (0.22, 0.28 nm) or more hydrated double helices (0.33 nm) along with local DNA denaturation (0.56 nm).

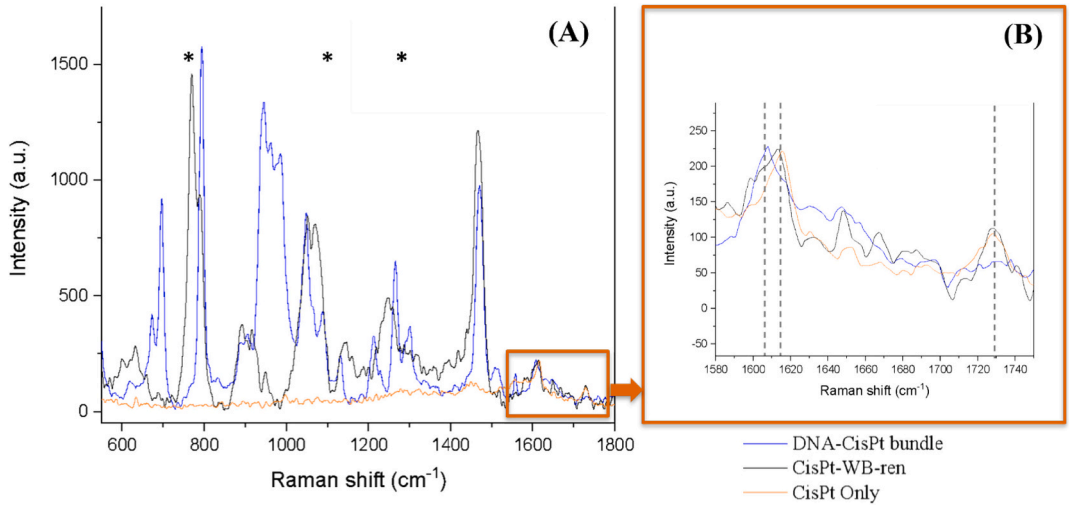


Fig. 3. Normalized Raman spectra of cisplatin aqueous solution, of cisplatin in a Tris-based buffer, and of a cisplatin-DNA suspended filament, showing the presence of the *chemotherapeutic* drug in all the samples reported. The spectra shown are normalized to the peak centered around 1610 cm^{-1} and related to cisplatin. (a) The buffer spectra show prominent peaks of the Tris cation (highlighted by asterisks and labels) with the characteristic main bands at 760, 1064, and 1467 cm^{-1} [66]. (b) The peak centered around 1730 cm^{-1} is present only when cisplatin is dissolved in solution and is not detected after its interaction with DNA. A shift in the band.

the region 2500–3750 cm^{-1}, Tris-related bands are centered around 3292 cm^{-1} and 3350 cm^{-1} (Fig. 4, Supporting Information) while no contribution from cisplatin is detected.

In our experimental conditions, the suspended sample is obtained from a starting solution composed by the cisplatin-DNA complex, monovalent cations (Na^+) and Tris-HCl buffering agent (Fig. 3, panels a, b). The spectra show the characteristic signature of the vibrations related to the DNA phosphate group, the deoxyribonucleic sugar, the bases [28] and Cisplatin [67]. It has to be noted that the spectra of the suspended cisplatin-DNA filaments are free from any contribution from Tris-related signature. In fact, during the dehydration process on SHS substrates, Tris cation are autonomously sieved out from the filaments, removing the non-interacted cations from the areas of investigation, as discussed in previous literature [45]. The suspended sample therefore is free from any additional signature contributions from the buffer which, in solution or deposited on flat substrates, overwrites most of the Raman features from the molecule.

With this premise, Raman peaks provide spectral information free

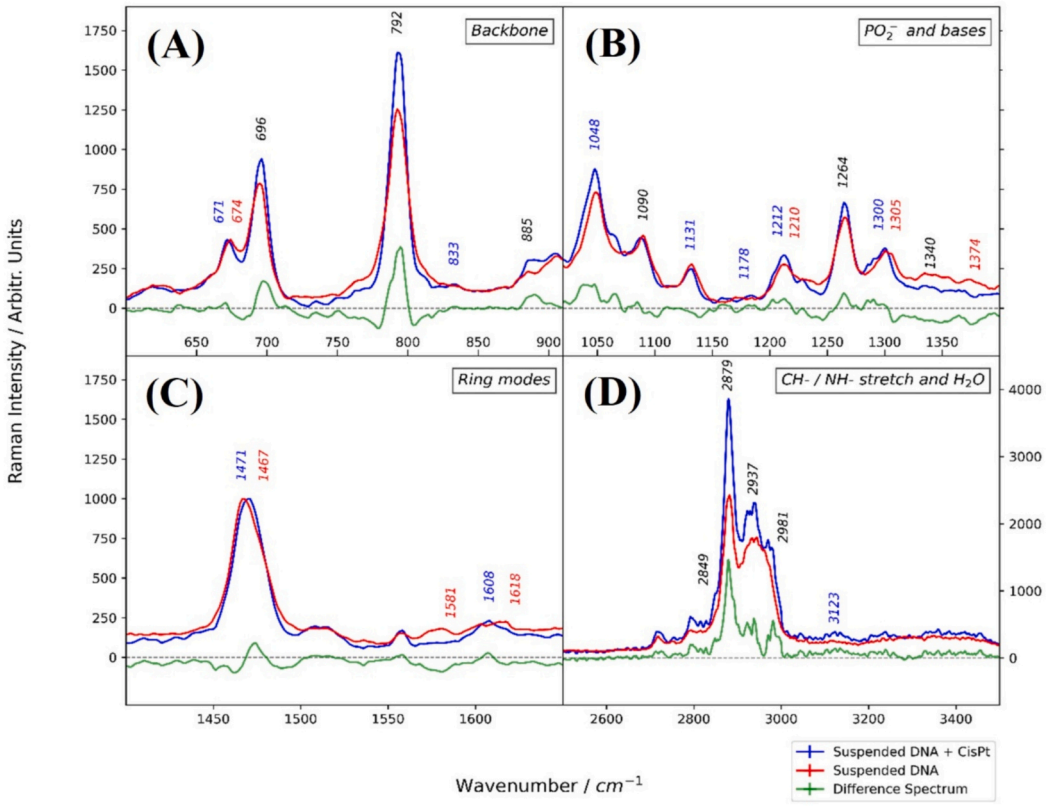


Fig. 4. Raman spectra of suspended cisplatin-DNA filaments (blue), free-standing pristine DNA (red), and their computed difference (green) in the range 600–920 cm^{-1} in panel a), 1020–1400 cm^{-1} in panel b), 1400–1750 cm^{-1} in panel c), and 2500–3750 cm^{-1} in panel d). The Raman spectra were normalized to a peak at 1468 cm^{-1} assigned to the 5'CH_2 scissor mode [45]. Peaks common to both spectra are labeled in black. Peaks labeled in blue and red correspond to suspended cisplatin-DNA and pristine DNA, respectively. The main spectral differences in terms of wavenumbers are reported as label on the graph. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from buffer excess, allowing the analysis only of the dry DNA filaments and the materials effectively interacting with the nucleotides.

3.3. Raman spectroscopy: Characterization of the cisplatin-DNA complex

The alterations of the DNA structure occurring upon the interaction with the chemotherapeutic compound were studied through the difference spectra (green line, Fig. 4 and Supporting Fig. 5) between the signature of the filaments treated with cisplatin and the suspended pristine DNA fingerprint after the renormalization to a reference peak (refer to Materials and Methods section). The deviation standard is indicated in supplementary Fig. 6 along with the averaged spectra. The spectral region 600–1650 cm^{-1} (Fig. 4a) showed details on the double helix form, sugar and bases conformation, and the phosphate backbone while the range 2500–3750 cm^{-1} (Fig. 4d) is related to the deoxyribosyl CH stretching vibrations and water contributions. The peak centered around 1610 cm^{-1} is assigned to cisplatin and its presence is the spectral confirmation of the drug integration into the double helix. To analyze the effects of cisplatin covalent binding in the DNA double helix a preliminary description of the spectral signatures of pristine suspended DNA filaments is provided, followed by the comparison with spectral information from cisplatin-DNA suspended filaments. In Table 1 are reported the main variations in Raman wavenumbers of the two compared samples and the related assignments.

3.3.1. Pristine suspended DNA

The peaks centered at 674 cm^{-1} and around 680 cm^{-1} are indicative of a C_2'-endo and a C_3'-endo conformation, respectively [20,21], and are related to the ring mode of guanine, used for the identification of DNA conformation. In this case the peaks identify DNA in the A and B-form, respectively. The suspended filament of DNA is composed of double helices arranged in concentrical layers [62]. The outer layers exposed to the environment previously showed features compatible with the A-DNA form [49] while the inner layers of the suspended material retain a 0.3 nm solvation layer [62]. The presence of different hydration levels of the filament are therefore reflected into the conformational details of the biomolecules studies [44].

The range 790–840 cm^{-1} describes the phosphate backbone stretching mode and allows distinguishing between different conformers of DNA. In our case, the bands centered at 792 cm^{-1} and the broad band at 834 cm^{-1} are related to B-DNA and can be compared to those reported for cisplatin-DNA adducts [22]. The two neighboring Raman bands at 788 cm^{-1} and 807 cm^{-1} are visible as shoulders of the main peak at 792 cm^{-1}. Despite the weak contribution, these bands are associated to the A-form of DNA as reported in previous literature related to DNA in hydrated gels or fibers [20]. Also, Lindsay et al. [34], demonstrated the decrease of the intensity of 807 cm^{-1} marker while diminishing the relative humidity. Deoxyribose vibrations are related to the presence of two bands at 896 cm^{-1} and 1047 cm^{-1} and related to deoxyribose and to C—O stretching [68].

The PO_2^- stretching mode in suspended pristine DNA samples is centered at 1090 cm^{-1} and is related to B-DNA [41,69,70], corroborated by the presence of the 834 cm^{-1} B-DNA marker band. The phosphodioxy vibration is also present at 1102 cm^{-1} and is diagnostic of an A-DNA vibration [71] of the outer layer of dehydrated DNA.

The vibrations related to the bases can be observed in the range 1100 cm^{-1}–1700 cm^{-1}. This range is informative about base pairing, as it depends on the electronic properties of the base residues. In our case, the most intense bands are assigned to (i) adenine, at 1210 cm^{-1}, 1305 cm^{-1}, 1343 cm^{-1}, and 1581 cm^{-1} [72] (ii) cytosine at 1264 cm^{-1} [20],

Table 1
This table reports on the main variations in Raman wavenumbers of suspended cisplatin-DNA with respect to pristine DNA suspended filaments taken as a reference. The assignments reported in the table refer to the four nucleotides (bases+sugar) adenine, thymine, guanine, and cytosine, A- and B-DNA conformers (A-DNA and B-DNA), phosphate (PO_2^-) group of the backbone, the deoxyribose sugar of the DNA backbone. sh, shoulder; vs, very strong; s, strong; m, medium; w, weak; vw very weak.

Cisplatin-DNA	Pristine DNA	Assignment
Wavenumber (cm^{-1})	Wavenumber (cm^{-1})	
671	674	Guanine ring breathing (A-DNA) [28]
780	780 (w)	Cytosine ring breathing [82]
792 (vs)	792 (s)	O—P—O single bond stretching vibration (B-DNA) [35]
–	804	Phosphate backbone main chain A-form [82]
834 (m)	–	Phosphate backbone main chain B-form [21]
885	885	Backbone deoxyribose [68]
902	902	Backbone deoxyribose [83]
930–1020	930–1020	Silicon secondary peak
1048 (vs)	1048 (s)	Backbone deoxyribose (vCO) [68]
1066 (sh)	–	Backbone symmetric CO stretching [83]
1088	1091	PO_2^- symmetric stretching [69]
–	1102	A helix PO_2^- [36]
–	1138	
1131	1132	C—C stretching [20]
1178	–	Unpaired thymine [76]
1203	–	Thymine, Adenine [79]
1212	1212	Thymine, Adenine [76]
–	1223	PO_2^- asymmetric vibration [69]
–	1236	Thymine, Cytosine [66]
–	1246	PO_2^- asymmetric vibration [73]
1265 (vs)	1265 (s)	Cytosine [84]
1286	–	Cytosine [85]
1290	–	C—C stretching
1300	1300	Adenine N7═C8 [75]
	1308	Adenine
1340	1340	Adenine, guanine C5—N7 [75]
–	1376	Thymine-Major groove marker peak [86]
1471	1467	Guanine [87]
1608	–	Cisplatin [65]
1618	–	GC carbonyl stretching [23]
2849	2849	
2879	2879	deoxyribosyl CH stretching vibrations
2937	2937	[20]
2981	2981	
3123	–	guanine C8—H stretching [80]
3237	3237	Water
3400	3400	

(iii) thymine at 1210 cm^{-1}, 1374 cm^{-1} and (iv) guanine at 1464 cm^{-1}, 1343 cm^{-1} and 1581 cm^{-1} [36]. The contribution of adenine and guanine overlaps at 1343 cm^{-1} and 1581 cm^{-1} [36]. The bands centered at 1223 and 1245 cm^{-1} are related to the PO_2^- asymmetric stretch vibration in pristine DNA [73].

The Raman large band centered around 1340 cm^{-1} is sensitive to adenine conformation and to the rearrangement of H-bonds at the acceptor site N7 of adenine. This band is split into two different peaks due to the mechanical coupling of the base with the sugar moiety: the peak at 1344 cm^{-1} indicates the presence of the C_2'-endo conformation, while the lower peak at 1332 cm^{-1} is associated with a less frequent O_4'-endo sugar pucker [72].

3.3.2. *Cisplatin-DNA suspended filaments*

Platination affects the nucleic acids spectra, and the predominant changes are observed for the backbone vibration, ring breathing, and stretching modes of adenine, guanine and thymine. The frequency at 671 cm^{-1} is characteristic of a C_2'-endo conformational isomer [20,21] and corresponds to a slightly red-shift with respect to pristine DNA (671 cm^{-1} from 674 cm^{-1}). The OPO single bond symmetric and asymmetric stretching vibrations are related to the band centered at 792 cm^{-1} and the broad band at 834 cm^{-1} and define a deoxyribose-phosphate main chain of a B-DNA conformer [35,74]. Both contributions are more intense in platinated DNA with respect to pristine DNA.

The phosphodioxy marker band (PO_2^-) in the suspended cisplatin-DNA sample is downshifted of 4 cm^{-1} (from 1091 to 1087 cm^{-1}) with respect to the suspended pristine DNA and indicates a relaxation of the double helix structure in accordance with previous literature [20]; this effect is supported also by the suppression of the phosphodioxy vibration at 1102 cm^{-1} (A-DNA), present only in suspended native DNA samples [71]. The bands related to the PO_2^- asymmetric stretch vibrations, present in pristine DNA are blocked under platination conditions [73].

The peaks at 1210 cm^{-1}, 1300 cm^{-1} and 1338 cm^{-1} correspond respectively to the adenine and thymine ring modes, to the coupled stretching vibrations of N7═C8, and to C5—N7 stretching localized on the purine ring of adenine [75]. The peak at 1210 cm^{-1} is slightly shifted to 1212 cm^{-1} and it is broadened towards 1204 cm^{-1}, as evident from the difference spectrum (Fig. 4b). A slight increase in the intensity of the signal at 1182 cm^{-1} is also detected. These combined findings assess the alteration of the A-T hydrogen bonding network with respect to pristine DNA. Also, similar assignments were previously described upon melting of A-T polynucleotides [76,77].

In the platinated sample, the whole band at 1340 cm^{-1} is less intense with respect to pristine DNA but centered on the same frequency, whereas the shoulder at 1305 cm^{-1} in pristine DNA it is red shifted to 1300 cm^{-1}. Moreover, it presents two shoulders, attributed to the superposition of guanine C5—N7 vibration, with the in-plane and out-of-plane NH_3 cisplatin deformation found in cisplatin-adenine crosslinks [65]. The 1374 cm^{-1} band, attributed to the CH_3 deformation in adenine, thymine, and guanine, is less intense in the drug treated sample while is present in the pristine DNA sample; a similar behavior has been reported for cisplatin binding to salmon sperm DNA [23,76,77]. The guanine band at 1471 cm^{-1} is blue shifted (4 cm^{-1}) while the peak at 1581 cm^{-1} is absent with respect to pristine DNA. These assignments are consistent with previously reported literature on DNA platination studies [13]. At last, the cytosine peak at 1264 cm^{-1} is more intense in the cisplatin treated sample. The peak intensity increase of the bands related to thymine and cytosine centered at 1212 and 1265 cm^{-1}, respectively, is in good agreement with previously reported in studies related to DNA melting and pre-melting transition and DNA packing and describes base unstacking [78,79].

Spectral differences between platinated and pristine DNA structures can also be detected in the region 2800–3000 cm^{-1} (Fig. 4, panel b) and related to the deoxyribosyl CH stretching vibrations [20,78]. In DNA exposed to cisplatin, the bands centered at 2849 and 2879 cm^{-1} showed higher intensity while the band between 2900 cm^{-1} and 3000 cm^{-1} is split into two contributions at 2937 cm^{-1} and 2981 cm^{-1} and associated to the less hydrated A-DNA conformer [20]. The band at 3123 cm^{-1} can be assigned to guanine C—H stretching [80].

The previous assignments endorse the conclusion that, regarding DNA conformation, a transition to a loosen state of DNA can be assessed, particularly through the Raman marker peaks sensitive to DNA conformation as the 671 cm^{-1} peak (guanine ring breathing), 792 cm^{-1} and 834 cm^{-1} peaks (phosphate backbone symmetric stretching), and the 1090, 1225, and 1245 cm^{-1} peaks (symmetric and antisymmetric PO_2^- vibrations). These findings are strongly supported by the HRTEM direct images acquired on the same samples. Moreover, ring vibrations are more intense in the platinated sample, suggesting the unstacking and an altered hydrogen bond network of the bases, as found in melting and pre-melting transitions [76,79,81]. Finally, it is confirmed that cisplatin binds preferentially to the guanine and adenine residues of DNA upon the formation of covalent bonds. Bands assigned to cytosine were not altered in terms of wavenumbers by drug administration, whereas the thymine-related bands were modified in both intensity and

wavenumbers [24,44].

4. Conclusions

It is well known that conformational transitions can occur in physiological conditions and depend upon hydration variations, solution and sequence composition [88,89] but can also reflect a non-physiological condition, precluding the correct functioning of the cellular machine [90–92]. In this work, we applied a quantitative method to unveil the integration mechanism of drugs into double helix and evaluate the structural effects of such modification to pristine DNA molecules. Data normalization allowed us to obtain a repeatable and quantitative analysis and directly comparing signals along the filament and between different filaments of the same device. Another key point in our study lies in the extended sampling of our analyte of interest: the presence of multiple filaments on the same device, arising from the same solution of analytes, led to uncovering fine variations in the structure of DNA upon cisplatin binding despite the small dimensions of the analyzed fibers. The genetic material did not need any purification or preliminary process to emerge from other interfering buffering agents or non-interacting molecules excesses. The simplified preparation strategy allows obtaining oriented filaments starting from biomaterials in solution in approximately 1 hour; sub molecular portions along the suspended filament have been co-localized and characterized by multiple techniques for a complete overview of the chemical and structural variations of the biosystem. The microRaman study on the DNA-SHS we proposed in this work demonstrated that the formation of cisplatin-DNA adducts causes helical distortion, detected with a conformational transition towards a more relaxed conformer of DNA and with the local denaturation of the double helix sequence. This evidence suggests that drug administration induces cytotoxicity through the alteration of DNA forms and consequently affecting transcription and replication. The case presented in this work relies on a well-known chemotherapeutic molecule such as cisplatin; we herein use a novel methodology to address biomedical questions and, to assess our method reliability. The promising results demonstrate how this approach is capable of showing how chemical modifications induce structural changes after drug administrations at sub molecular level. It is therefore a strong candidate platform to study the effects of unknown drugs.

Ethics approval statement

No ethics approval was required for this work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2025.126606.

Data availability

Data will be made available through Zenodo repository.

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