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# Raman on suspended DNA: novel super-hydrophobic approach for structural studies.

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**Keywords**: DNA, A-form, B-form, super-hydrophobic devices, Raman spectroscopy.

**Abstract**

The A- and B- form are two of the most common structural conformations of double strand DNA present in nature and they can interchange on the basis of the helices hydration [1,2]. Herein we demonstrate that the use of non-destructive techniques such as Raman spectroscopy coupled with the use of a super-hydrophobic device, allows the clear identification of the DNA hydration state, of the backbone (phosphate + deoxyribose sugar) conformation and of the nucleotides. There is a wide prospect for an increase of knowledge in biomolecules using this combined approach resulting in a significant impact in the study of more complex supramolecular assemblies and of fine chemical variation along the genomic loci undergoing to epigenetic variations.

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## 1. Introduction

Deoxyribonucleic acids (DNA) conformation and topology are fundamental characteristics for the interaction with other molecules such as selective DNA-binding proteins [3]. In nature DNA can assume a variety of structural forms. A- and B- forms represent the major ones, both stabilized by hydrogen bonds between base pairs; they differ by several structural parameters and they can interchange due to the intrinsic flexibility of the molecule and depending on the hydration status of the helix. As obtained in the 50s by X-ray crystallography, above 75% of relative humidity the B-form is dominant; below that value, the A-form is favourite [1,2,4]. The biological function of the macromolecule remains the same, while the environmental contribution affects its structural features. If compared to the B-form, A-DNA is smaller: the pitch and diameter of the helix decrease from ~36 Å to ~27 Å and from 23.7 Å to 21.2 Å. The tilt of the base pairs, with respect to helix axes, changes from -1º to 19º while the repeating units are less rotated, becoming 32.7˚ from the 35.9˚ of the B-DNA (**Supplementary Figure 1**) [1,2,4–6].

These characteristic features can be observed using non-destructive techniques such as crystallography, sensitive to the molecular conformation. Among these approaches, Raman spectroscopy is widely used for the study of a wide range of analytes and biological samples, from macromolecules [7–10] to the entire cell [11]. With respect to the traditional approach relying on crystallography, this new combination of techniques opens the possibility to focus an a reduced set of molecules at the time, detecting local conformational changes.

Recently, the investigation of diluted molecules has been exploited coupling the use of spectroscopy and super-hydrophobic surfaces (SHS) [12–14]. Silicon-based SHS comprising periodic hexagonal or circular lattices of micro-pillars of different diameter and pitch have been previously investigated and applied. A small drop of solution containing a diluted molecule of interest is deposited over the micro-structured substrate and let evaporate until the sample is completely dry. During the dewetting process, the quasi-spherical shape of the drop is maintained while the volume is reduced, concentrating the molecules suspended in the solvent. At the end of the process the molecules accumulate [12,15] and/or suspend [6,16,17] over a precise area of the device and the solvent residual is confined in the point where the evaporation ends.

In this work we propose a new reproducible approach to study the coupling of Raman spectroscopy as non-destructive technique and the super-hydrophobic properties of a micro-structured device in terms of the conformational status of DNA and more precisely the presence of the A- or the B-form in a suspended bundle of DNA.

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## 2. Materials

### 2.1 Super-hydrophobic device realization

Si-based micro-pillars devices were prepared by means of a combination of optical lithography and deep reactive ion etching (DRIE). In the first step, the pattern of pillars was defined by means of a combination of positive optical lithography and metal deposition via sputtering, followed by lift-off in acetone. A thin layer of Ti (10 nm) was firstly deposited over Si to promote the adhesion of a Au layer (50 nm). A subsequent deposition of Cr (50 nm) was performed to protect Au during DRIE. The sample was then etched in a DRIE system to define the pillars, with a final height of the structures of about 10 μm. Finally, the residual protecting layer of Cr on top of the pillars was removed with a selective wet etching. The surface structured in this way required a further functionalization with hydrophobic materials to make it super-hydrophobic. This was achieved with functionalization with Perfluorodecyltrichlorosilane (FDTS). The device was then ready to be employed for sample deposition.

### 2.2 Sample preparation

Lambda DNA (New England Biolabs, NEB UK) was diluted in a saline buffer properly titrated to obtain thin and purified suspended DNA strains. For this purpose, the Lambda DNA stock solution was resuspended in a buffer containing a 6.5 mM NaCl and 10 mM Tris-HCl, pH 9.3 and preheated as previously reported [6]. 5 μl of the freshly prepared solution was deposited on the super-hydrophobic device placed on a hot plate at 25˚ [16] until complete evaporation of the solution.

### 2.2 Characterization of the DNA samples

The result of the evaporations were investigated with a WITec confocal Raman system, equipped with a 532 nm laser and a 100x objective working at a laser power of 2.2 mW, with an exposure time of 1 s and with 50 accumulations. Seven spectra obtained by measurements performed on 7 different bundles of the same device were averaged to obtain the spectra analysed in this work. Measurements were performed at 21˚C and at RH 53%. Samples were then sputter coated with 2 nm of Iridium (Q150T sputter coater, Quorum Technologies) and imaged by SEM (FEI, Quanta 200) working at an acceleration voltage of 3 kV.

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## 3. Results and discussion

In this work we propose the use of Raman spectroscopy to characterize the conformation of a bundle of suspended DNA, determining if the helix arrangement is in the A- or in the B-form. As previously reported [6,12–14,17–19], single droplets of a diluted solution of lambda DNA can be deposited on super-hydrophobic devices, specifically micro-fabricated silicon pillars arranged in a circular pattern. The same design is herein used for the suspension of DNA bundles that undergo to Raman investigation. The evaporation of the buffer concentrates the solution, shrinking the droplet towards its centre: in this movement, the DNA molecules linked to a pillar are pulled to the neighbour one. In **Figure 1** is reported a sketch of the general scheme of the approach used in this work including the molecule deposition process and the conformation assumed on the pillars after drying, as well as the setup used for the measurements.

In **Figure 2** are reported the results of the DNA solution droplet dehydration **Figure 2a** shows residuals of non-suspended DNA and buffer salts, by design concentrated in a localized area of the sample corresponding to the final drying point of the evaporation process, providing an additional effective sieving system for the sample. **Figures 2b-d** show bundles of suspended DNA at different magnifications, imaged after a thin iridium coating (2 nm). The dsDNA bundles, guided during the evaporation process by the circular pattern of the device, resulted in a highly ordered arrangement between micro-pillars. The molecules are suspended over a macroscopic area with a uniform and well defined distribution. The diameter of the bundles, in fact, ranges from 8 nm (coating included) in correspondence to the dehydration starting point to 50 nm close to the center, demonstrating the efficiency of the device in the controlled placement and confinement of the sample in a precise and ordered pattern. This results are in good agreement with the previously published data [6,9] in which the DNA strands are suspended in radial manner over the super-hydrophobic device. Uncoated samples were considered to perform the micro Raman analysis of the DNA bundles (**Figure 3**). Raman spectra of DNA fibers were acquired in the 450-3750 $cm^{-1}$ spectral range. The clear contribution of the symmetric and asymmetric vibration of the C-H bond in the methyl groups is assigned to the 2900-3000 $cm^{-1}$ spectral range. In the range 600-1700 $cm^{-1}$ several characteristic features of nucleic acids were observed. Bands in the regions 250-800 $cm^{-1}$, 800-1150 $cm^{-1}$ and 1150-1700 $cm^{-1}$ are generally sensitive to nucleoside (sugar + base) conformation, backbone (phosphate + base) geometry and bases structure, respectively [20–24]. The data reported in **Figure 3a** is relative to an average over 7 different spectra, shown to highlight the distinctive features in DNA. A broad peak in the region 600-700 $cm^{-1}$, is selected as a benchmark of the conformational changes in the

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DNA double helix is due to the overlapping of A- and B-form bands. The analysis of two single scan measurements (**Figure 3b,c**) revealed the isolated fingerprint of the DNA in its B-form (**Figure 3b**) and in the A-form (**Figure 3c**), with bands centred at 688 cm-1 and 670 cm-1, respectively. These wavenumbers are in accordance with previously published data [21–23]. A detailed overview of the peaks and their tentative assignment are reported in **Table 1**.

Despite the supposed dehydrated condition of the suspended bundle, Raman spectra showed vibrational bands related to B-DNA in the region 600-700 $cm^{-1}$. We hypothesize that this strong B-form contribution is given by the hydrated status of the helices hidden in the internal part of the bundle. The bundle in fact is a superstructure composed by a central DNA helix and several layers of other helices that can retain a hydration shell. Notice that the peak at 521 $cm^{-1}$ and the 940-980 $cm^{-1}$ region are related to the primary and secondary peaks of the silicon device used in this work.

## 4. Conclusions

In this work we reported preliminary results on a study of conformational changes of DNA by means of non-destructive Raman analysis coupled to a proper preparation protocol and micro-fabricated engineered super-hydrophobic substrate, designed and optimized for this purpose. The protocol allows obtaining uniformly arranged DNA bundles on macroscopic areas while maintaining the biological matter in a physiological compatible working condition. The presence of nucleotides, phosphodiester bonds and the backbone is clearly appreciable. Data show that the bundles have a strong B-form behaviour. This statement is supported by direct HRTEM imaging. Previously published results showed a structure of the helix at the edge of the DNA bundle that is compatible with the geometrical features of the A-form [6]. In our hypothesis, this superposition of the two different forms of DNA towards the centre of the bundle might be at the origin of the complex behaviour of the HRTEM interference pattern obtained in this area [6].

These preliminary results are extremely promising in perspective of a non-destructive characterization [8,12] of single bases variation and epigenetics mutation of nucleic acids as well as protein/DNA interaction by means of an easy-to-implement Raman characterization. The effect of different ion type and concentration in different buffers, yet under physiological conditions, results in appreciable variations in deposits geometry and conformation allowing for fine control on the final result.

The method we propose, *i.e.* the controlled and engineered deposition of single suspended bundles with tailored substrate geometry, allows investigating a very wide range of biological variations in their natural environment improving the intrinsic sensitivity of the Raman technique on tiny structural and geometrical changes in the molecules.

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We expect that the protocol herein proposed in the near future will allow systematic studies in different fields, spanning from DNA/protein interactions to epigenetic mutations with promising application in early diagnostics.

## 5. Acknowledgments

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**Captions**

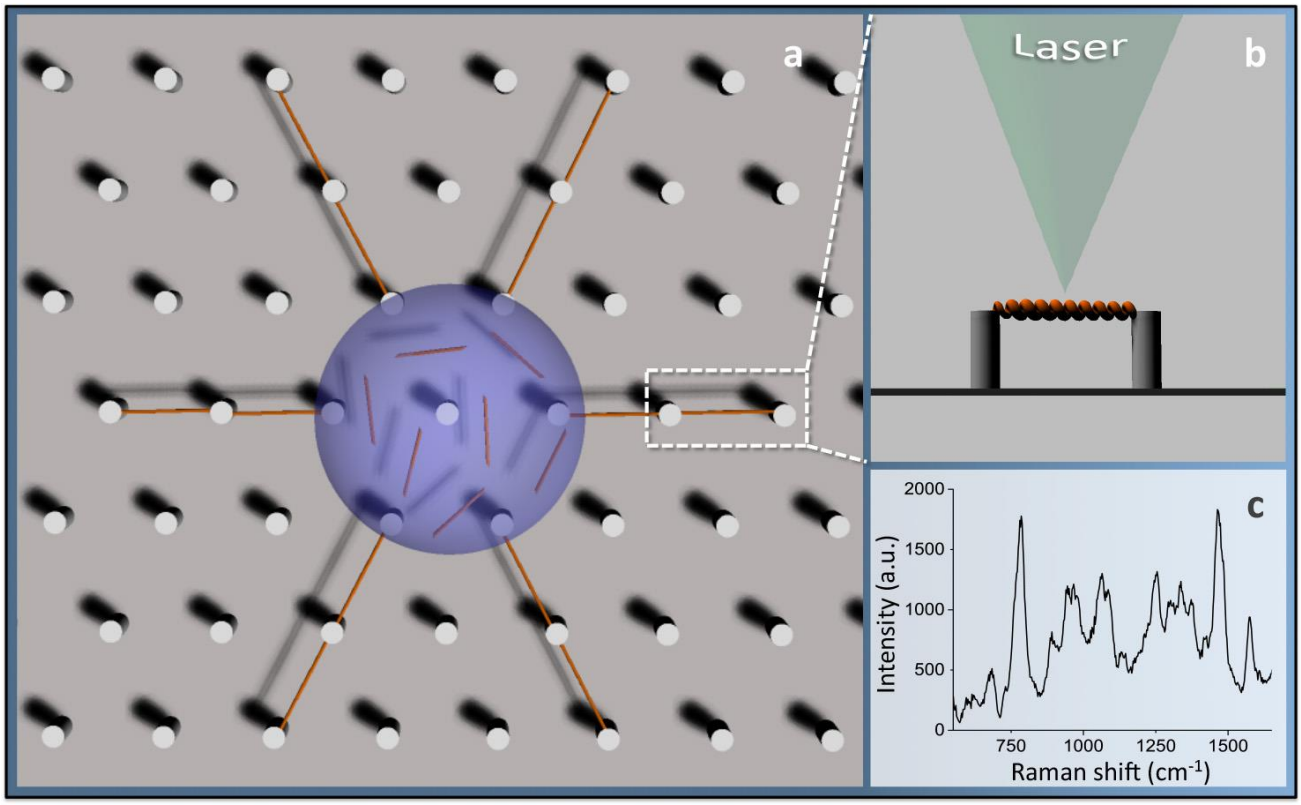


**Fig. 1. General scheme and results of the evaporation process. a**) A micro pillar patterned surface permits the evaporation of a millimetric droplet of saline solution containing dsDNA molecules [1]. **b**) DNA bundles suspended between micro pillars were obtained as droplet dehydration result and **c**) were characterized by means of Raman spectroscopy.

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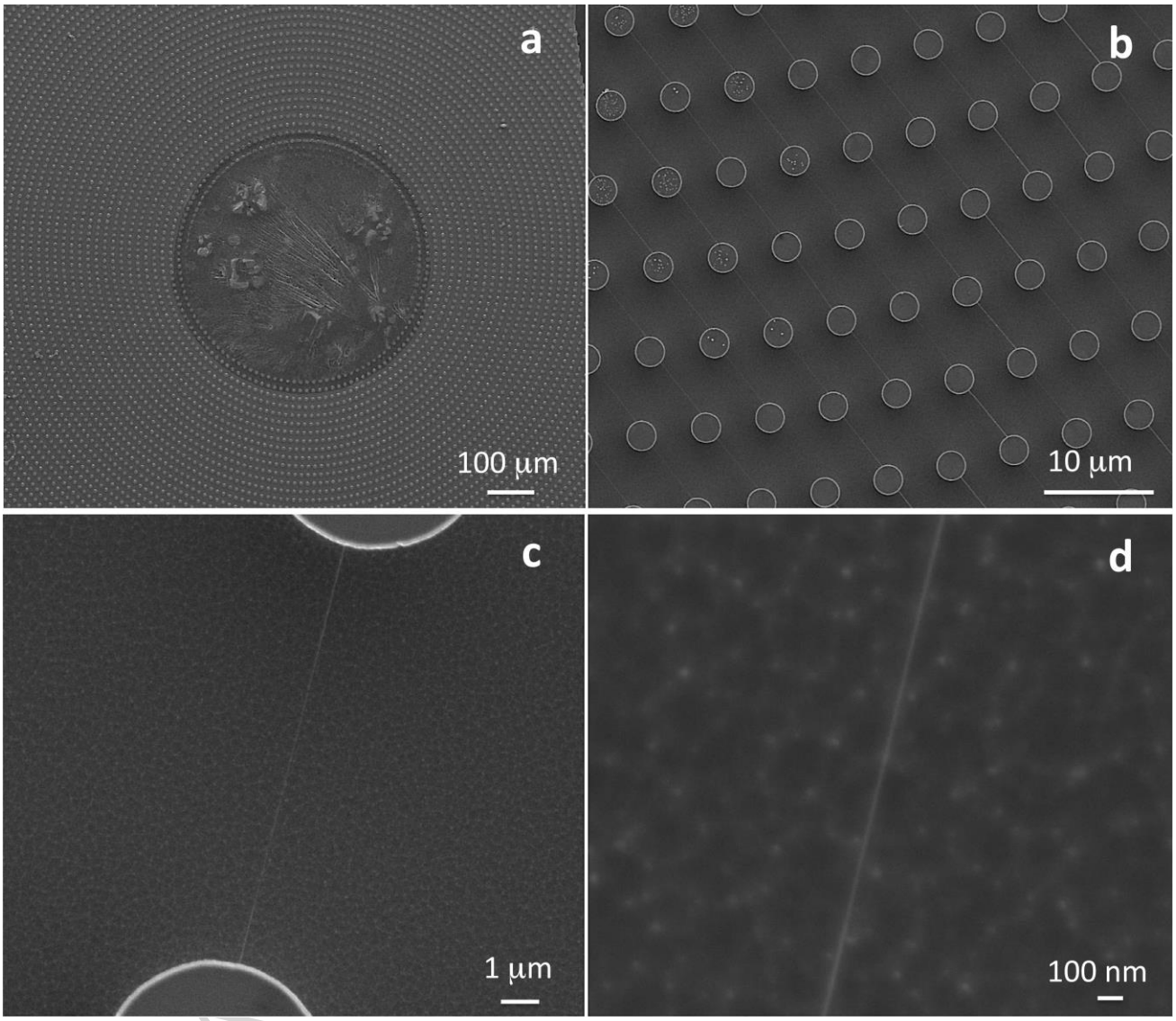


**Fig. 2. SEM imaging of suspended dsDNA. a**) Low magnification image of the sample after complete evaporation of the solvent. Salts removed from the bundles are confined in a well-defined area of 500 nm diameter, corresponding to the dehydration end point; out of this area for an extension of 300 nm, the dsDNA bundles are suspended between the top flat part of adjacent cylindrical micro-pillars (height 10 μm, diameter 6 μm, gap 12 μm). **b**) Micrographs at higher magnifications showing a regular and homogenous array of dsDNA bundles over a macroscopic area, demonstrating the efficiency of the device in confining and distributing the sample in a well-defined arrangement. The uniform thickness of the bundles in (**c, d**) over the entire length indicates the absence of salt contamination which strongly contributes to the final control on the desired diameter.

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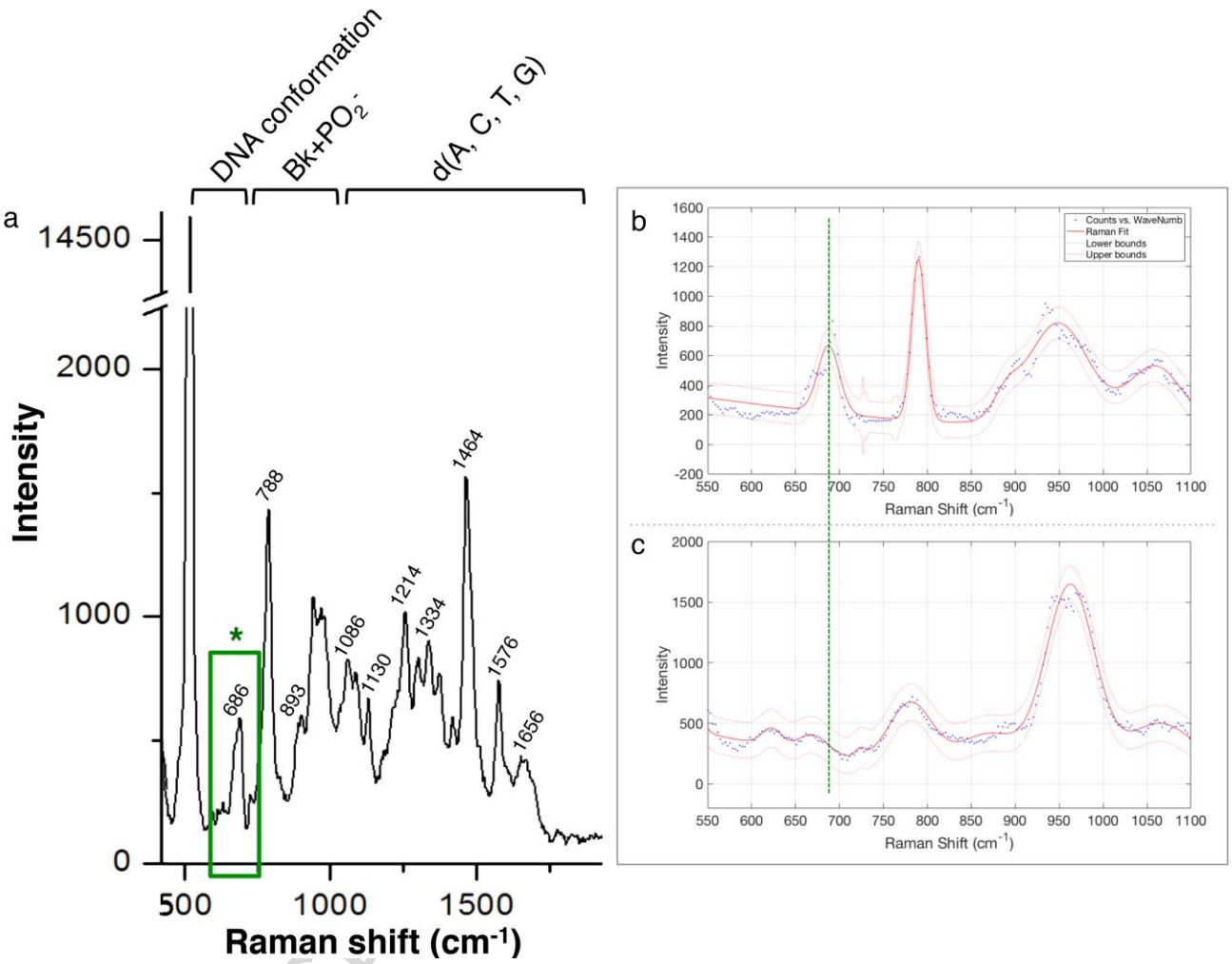


**Fig. 3. Raman spectra of a dsDNA suspended fiber in the 450-3750 cm$^{-1}$ range. (a)** The top labels indicate the characteristic spectral ranges of DNA. In this work we focused on the region 600-700 cm$^{-1}$, indicated by the green box and the asterisk and sensitive to conformational features. Raman frequencies of prominent bands are listed and clarified in **Table 1**. Two separate spectra regarding the **(b)** B-form and the **(c)** A-form are reported; The Raman fits clearly show bands centred at 688 cm$^{-1}$ and 670 cm$^{-1}$.

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| **Wavenumber ($cm^{-1}$)** | **Assignment** [20–23,25] |
|---|---|
| 686 | B-form |
| 725 | dA |
| 788 | bk |
| 893 | d |
| 942-980 | Silicon |
| 1086 | $PO^{2-}$ symmetric stretching |
| 1130 | ν(C-C) |
| 1214 | dC, dT |
| 1258 | dA, dC |
| 1301 | dA |
| 1334 | dA, dG |
| 1373 | dA, dT, dC |
| 1418 | d[δ(C5`$H_2$)], dA |
| 1464 | d[δ(C5`$H_2$)] |
| 1576 | dG, dA |
| 1658 | dT |

**Table 1. Characteristic Raman DNA bands with the correspondent assignment.** The assignments are indicated as follows: adenine (A), thymine (T), guanine (G), cytosine (C), backbone (bk), deoxyribose (d), ν (stretching), δ (deformation).

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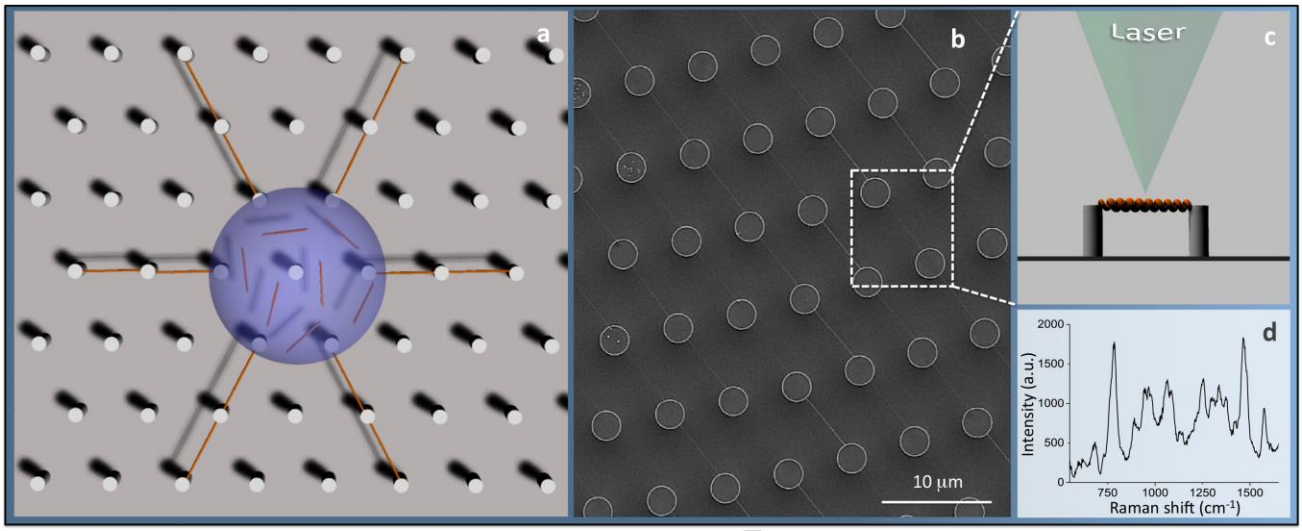


Graphical abstract

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*Highlights*

Raman on suspended DNA: novel super-hydrophobic approach for structural studies.

Monica Marini[a], Marco Allione[a], Bruno Torre[a], Manola Moretti[a], Tania Limongi[a], Luca Tirinato[a], Andrea Giugni[a], Gobind Das[a], Enzo di Fabrizio[a,*]

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1. Non-destructive Raman spectroscopy technique coupled with super-hydrophobic devices for biological fibers investigation
2. Super-hydrophobic surfaces comprising micro-pillars for DNA localization
3. Discrimination between A- and B-form of dsDNA suspended between micro-pillars by means of Raman analysis.
4. Electron Microscopy preliminary investigation of suspended dsDNA molecules

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