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# Energy Thresholds of DNA Damage Induced by UV Radiation: An XPS Study

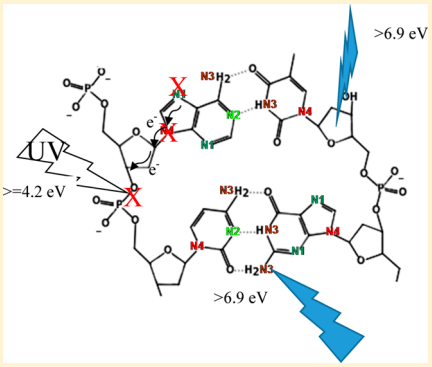
P. J. Gomes,[†] A. M. Ferraria,*[,‡] A. M. Botelho do Rego,[‡] S. V. Hoffmann,[§] P. A. Ribeiro,[†] and M. Raposo*[,†]

[†]CEFITEC, Departamento de Física, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

[‡]Centro de Química-Física Molecular and IN, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal

[§]ISA, Department of Physics and Astronomy, Aarhus University, Ny Munkegade 120, Building 1520, DK-8000 Aarhus C, Denmark

**ABSTRACT:** This work stresses on damage at the molecular level caused by ultraviolet radiation (UV) in the range from 3.5 to 8 eV, deoxyribonucleic acid (DNA) films observed by X-ray photoelectron spectroscopy (XPS). Detailed quantitative XPS analysis, in which all the amounts are relative to sodium—assumed not to be released from the samples, of the carbon, oxygen, and particularly, nitrogen components, reveals that irradiation leads to sugar degradation with CO-based compounds release for energies above 6.9 eV and decrease of nitrogen groups which are not involved in hydrogen bonding at energies above 4.2 eV. Also the phosphate groups are seen to decrease to energies above 4.2 eV. Analysis of XPS spectra allowed to conclude that the damage on bases peripheral nitrogen atoms are following the damage on phosphates. It suggests that very low kinetic energy photoelectrons are ejected from the DNA bases, as a result of UV light induced breaking of the phosphate ester groups which forms a transient anion with resonance formation and whereby most of the nitrogen DNA peripheral groups are removed. The degree of ionization of DNA was observed to increase with radiation energy, indicating that the ionized phosphate groups are kept unchanged. This result was interpreted by the shielding of phosphate groups caused by water molecules hydration near sodium atoms.




## INTRODUCTION

As a result of many investigations about the effect of radiation in biological systems, namely in living cells and also on biological molecules such as DNA, it is well established that when primary ionizing radiation, as $\beta$, $\gamma$, UV, X-rays and cosmic rays reach matter, a shower of excited and ionized molecules and secondary or even thermalized electrons are generated with energies below the ionizing energies. With respect to electrons, although they have short lifetimes,[1] normally within 1 ps before being solvated, they can induce irreversible cell damage such as DNA strand breaks, changes in nucleobases, sugar destruction, cross-linking, and dimer formation, among others[2] and references therein.

Low-energy radiation, as ultraviolet (UV), is also known to cause DNA damage in the cellular environment, with severe implications in cell-acting and promoting, in certain cases, the germination of cancer cells.[3−6] Several studies have been conducted toward the understanding of the chemical and physical processes taking place at the molecular level, when a DNA molecule, outside the cellular environment is subjected to low-energy particles or radiation.[7−17] These studies revealed that low-energy particles and radiation cause DNA changes or removal of molecular groups[12] and both single and double DNA strand formation.[9] For example, phosphate groups were seen to be removed from DNA cast films irradiated with 4.88 eV light, with consequent reduction of DNA chain conductivity, which comes from electron hopping between base-pairs and phosphate groups.[18] Despite several studies carried out making use of different approaches and techniques,[19−26] the damage mechanisms taking place at the different groups of the DNA molecule itself (i.e., outside of the cell environment), when submitted to low-energy radiation are fairly well-understood, namely, with respect to strand breaks discrimination. However, there are still few studies on the full range of UV covering the range of a few to 10 eV due to the complexity of the DNA molecule and the surrounding biological environment. For this reason, most of the data on the effect of radiation or particle beams has been obtained on DNA constituents, with only few works dedicated to this effect on DNA molecule as a whole and even less to DNA within aqueous environments as achieved in biomimetic rudimentary cells.

Calf thymus DNA is commercially available and was shown to be easily processed in thin film form onto solid supports,[13] which are stable under ultrahigh vacuum conditions, making it adequate to address damage studies in condensed phase. In the UV range of 110 to 300 nm, the calf thymus DNA films present a vacuum ultraviolet absorption spectrum,[13] which exhibits absorption features that can be assigned as follows: the band at 263.4 nm (4.71 eV) was assigned to excitations in all the bases[27] and to the anionic phosphate esters first ionization;[28]

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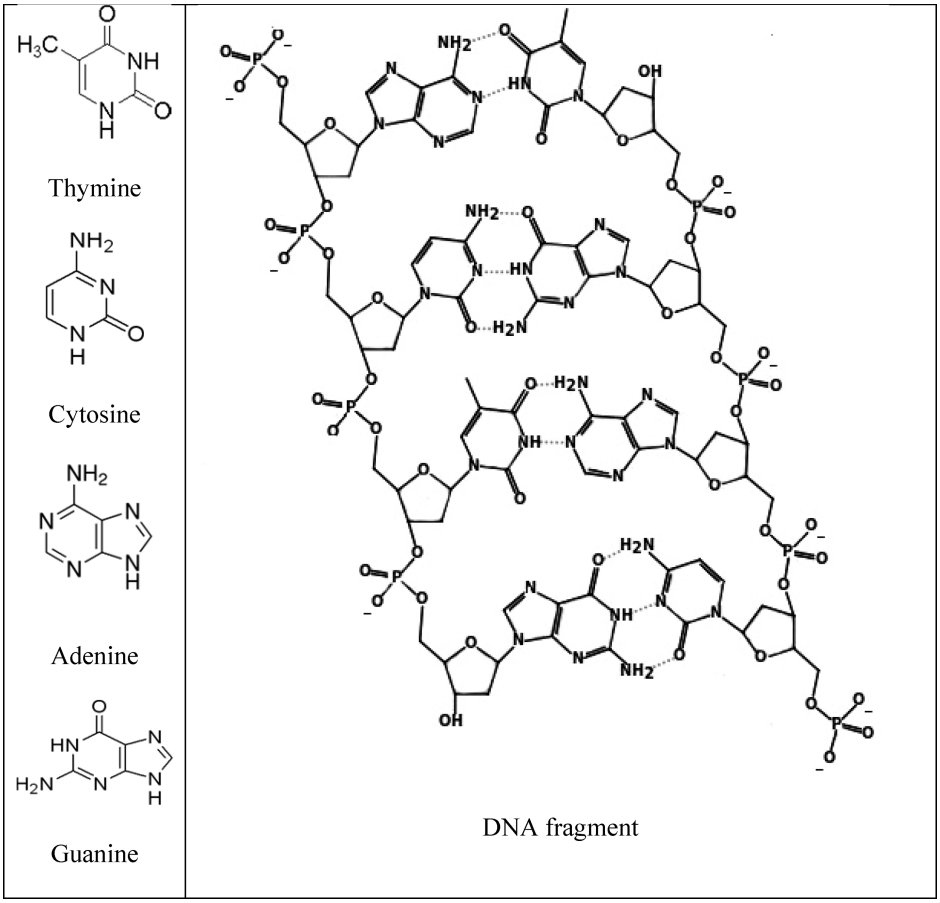


**Figure 1.** DNA bases chemical structures on the left and a schematic representation of a fragment of a double strand DNA molecule.

the peak at 209.9 nm (5.91 eV) was considered to be due to n → $\pi^*$ guanine transition and to a $\pi \rightarrow \pi^*$ transition in thymine;[29] the peaks at 177.4 nm (6.99 eV), 188.3 nm (6.58 eV), and 201.5 nm (6.15 eV) were referred to the strong transitions at 6.81 eV ($8^1$ A'), 6.38 eV ($6^1$ A'), and 6.28 eV ($4^1$ A'), for purine N(7)H and N(9)H;[30] the peak at 188 nm was also associated with the $\pi \rightarrow \pi^*$ transition of the C═O chromophore in the sugar open-chain;[31] a peak at 161.8 nm (7.66 eV) was considered arising from strong thymine absorption as determined by Shukla and Leszczynski;[29] and a peak centered at 119.8 nm (10.35 eV) was attributed to direct ionization of nucleobases.[32] In this work, to better understand the damage caused by different radiation energies, namely to find damage thresholds, damage on calf thymus DNA films caused by exposure to a vacuum UV radiation beam in the range of 300 nm (4.13 eV) to 160 nm (7.75 eV) was investigated using XPS. From both qualitative (binding energies) and quantitative analysis (peak area combined with sensitivity factors), as a function of the radiation energy, the DNA damage thresholds will be obtained from XPS spectra analysis and correlated with the UV−vis absorption spectrum.

## EXPERIMENTAL DETAILS

Cast films were prepared using DNA sodium salt from calf thymus (DNA), obtained from Aldrich. DNA was dissolved in pure water, obtained from the Millipore Milli-Q system (resistivity ≥ 18.2 MΩ cm), to a concentration of 0.5 mg/mL and cast onto silicon substrates which were previously hydrophilized in a Piranha solution, $H_2SO_4/H_2O_2$ (7:3), for 10 min. The films were dried for 2 h in vacuum desiccators and exposed to a $1.14 \times 10^{15}$ photons VUV radiation beam in the range of 160 to 300 nm, from the Astrid Synchrotron radiation source (UV1) facility at Aarhus University, Denmark.[33,34] The setup consisted of a vacuum chamber containing a sample holder. The wavelength was selected by means of a toroidal dispersion grating placed upstream of the sample. The typical resolution was better than 0.08 nm. Since all samples have been submitted to ambient light conditions during handling, nonirradiated samples will be considered as submitted to 340 nm wavelength radiation for graphical representation purposes. Consequently, data relative to nonirradiated samples will be designated in the Results and Discussion as irradiated at 340 nm wavelength radiation.

The characterization of DNA samples was performed in an X-ray photoelectron spectrometer XSAM800 (KRATOS) operating in the fixed analyzer transmission (FAT) mode,[35] with a pass energy of 20 eV, a power of 60 W (= 5 mA × 12 kV) and the nonmonochromatised Al K$\alpha$ X-ray (h$\upsilon$ = 1486.6 eV) source. All the samples were analyzed on the central part of the sample (i.e., over a 1 × 3 $mm^2$ spot area at an angle of 0° with respect to the sample surface normal using the high-magnification mode). The measurement time was similar for all the samples. Spectra were recorded with a Sun SPARC Station 4 with Vision software (Kratos) using a 0.1 eV step. X-ray source satellites were subtracted, Shirley background and pseudo-Voigt profiles (Gaussian-Lorentzian products) were fitted to each region using a nonlinear least-squares algorithm.[36] No charge compensation (flood-gun) was used. Binding


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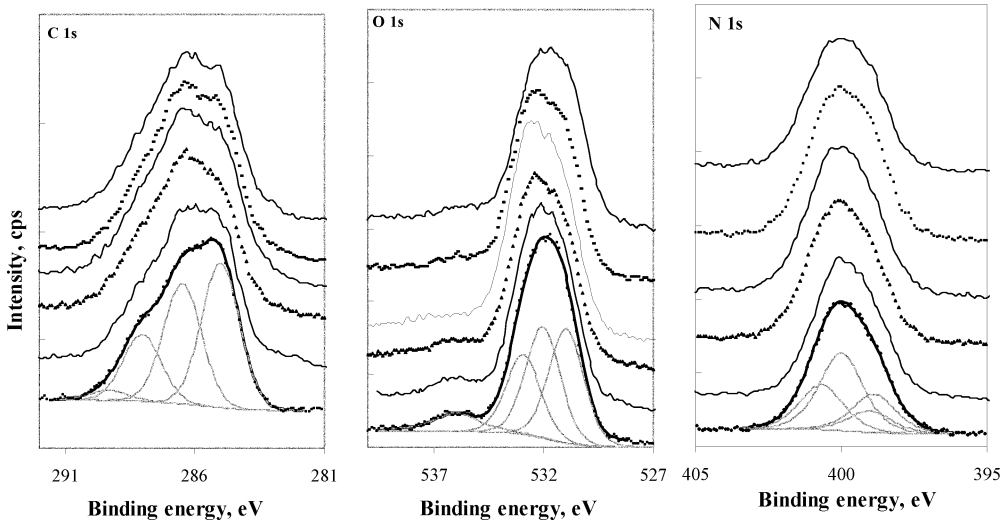


**Figure 2.** C 1s, O 1s, and N 1s XPS spectra obtained at takeoff angle of 0° with respect to the normal to the surface, for DNA samples, from bottom to top, irradiated with 160, 180, 200, 220, and 300 nm UV radiation. The top curves correspond to the nonirradiated sample spectra. For the sake of simplicity, only the curve-fitting for the sample irradiated with 160 nm radiation is shown. Curves were set off along the *y* axis.

**Table 1. Elemental Composition in Percentage, Obtained from XPS Spectra Taken at a Take-Off Angle of 0° Relative to Normal of DNA Cast Films Surface, Irradiated with $1.14 \times 10^{15}$ Photons UV Beam at Different Wavelengths[b]**

| wavelength (nm) | 160 | 180 | 200 | 220 | 300 | 340[a] | assignment |
|---|---|---|---|---|---|---|---|
| C1 | 22.0 | 20.6 | 17.9 | 18.7 | 19.8 | 19.8 | C−C, C−H |
| C2 | 18.3 | 19.9 | 21.1 | 20.4 | 21.0 | 20.7 | C−N, C═N, C−O |
| C3 | 10.2 | 9.9 | 10.2 | 9.6 | 9.5 | 9.8 | N−C═O, N−C═N, $C(N)_3$, N−C−O |
| C4 | 1.5 | 1.5 | 1.8 | 1.9 | 1.9 | 2.2 | N−(C═O)−N |
| total C | 52.0 | 51.9 | 51.0 | 50.6 | 52.2 | 52.4 | |
| O1 | 10.7 | 9.8 | 9.5 | 9.8 | 9.9 | 10.5 | aromatic O═C |
| O2 | 10.4 | 9.9 | 8.4 | 7.7 | 6.7 | 7.5 | $PO_x$ + Aliph. O═C |
| O3 | 7.4 | 8.9 | 10.0 | 11.8 | 10.4 | 9.5 | $PO_x$ + O−C |
| O4 | 1.0 | 0.7 | 0.9 | 0.6 | 0.6 | 0.5 | $H_2O$ |
| total O | 29.5 | 29.2 | 28.8 | 29.9 | 27.6 | 28.0 | |
| N1 | 2.7 | 2.9 | 3.3 | 3.0 | 4.0 | 3.8 | see text and Figure 3 |
| N2 | 1.5 | 1.5 | 1.9 | 1.9 | 1.9 | 1.9 | |
| N3 | 5.5 | 5.9 | 5.8 | 5.9 | 5.0 | 4.7 | |
| N4 | 3.2 | 3.2 | 3.5 | 2.9 | 3.8 | 3.8 | |
| total N | 12.9 | 13.5 | 14.5 | 13.8 | 14.7 | 14.2 | |
| P $2p_{3/2}$ | 1.5 | 1.7 | 2.0 | 1.9 | 2.2 | 2.1 | phosphate |
| P $2p_{1/2}$ | 0.9 | 0.9 | 1.0 | 1.0 | 1.1 | 1.1 | |
| total P | 2.4 | 2.6 | 3.0 | 2.9 | 3.2 | 3.2 | |
| Na 1s | 3.2 | 2.8 | 2.8 | 2.8 | 2.3 | 2.3 | $Na^+$ |

[a]Sample not irradiated but submitted to ambient light conditions during handling was considered irradiated at 340 nm. [b]Relative errors are estimated to be less than ±10% for components and less than ±2% for the total.

energies (BE) were corrected by setting the lowest C 1s BE to 285.0 eV.[37] For quantification purposes, sensitivity factors were 0.66 for O 1s, 0.25 for C 1s, 0.42 for N 1s, 0.39 for P 2p, and 2.3 for Na 1s. The chemical structure of the DNA bases and a schematic DNA molecule fragment are displayed in Figure 1.

## 3. RESULTS AND DISCUSSION

**3.1. XPS Characterization.** A survey over XPS spectra of UV irradiated DNA films at different wavelengths revealed the presence of carbon, oxygen, nitrogen, phosphorus, and sodium. The detailed spectra of carbon, oxygen, and nitrogen presented several components, as shown in Figure 2 (panels a, b, and c), respectively, where the pseudo-Voigt profiles used to fit experimental peaks, for the sample irradiated with 160 nm wavelength UV radiation, have also been included.

The XPS C 1s region was fitted, for all the samples, with four components centered at 285.0 (C1, used as charging correction reference), 286.5 ± 0.1 (C2), 288.1 ± 0.1 (C3), and 289.3 ± 0.2 eV (C4). The first one is assignable to carbon in C−C or C−H and the last one to N−(C═O)−N groups. The two intermediary peaks correspond to a mixture of functions which should have binding energies around those two values. By increasing binding energy order, should correspond to the groups: C−N, C═N, C−O, N−C═N, N−C−O, N−C═O, and $N{=}C(NH_x)_2$. These peaks are in accordance with literature.[37−39] The computed atomic percentages for those components are displayed in Table 1.

The O 1s peak was fitted with four components: one centered at 531.0 ± 0.1 eV (O1) assigned to O═C bonds in an aromatic system; a second one centered at 532.1 ± 0.2 eV



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(O2), assigned to both O in phosphate group and carbonyl in an aliphatic chain; a third one centered at 532.9 ± 0.1 eV (O3), assigned to O in O−C bonds; and a fourth one centered at 536.1 ± 0.2 eV (O4) assignable to water entrapped in the film.[40] For the N 1s fitting, the following methodology was used: assuming that an equal number of AT and GC pairs exist in the DNA here analyzed, four different nitrogen species were identified in the ds-DNA. By increasing binding energy order: $sp^2$ (imine nitrogen), donor $sp^2$ (imine nitrogen acting as donor in hydrogen bonding), acceptor $sp^3$ (amine nitrogen acting as electron acceptor in hydrogen bonding), and $sp^3$ (amine nitrogen). They were labeled from N1 to N4, respectively. To better-understand the nature and the stoichiometric ratio of these components, Figure 3 displays the position and the number of each of the species.

Therefore, the atomic ratio N1:N2:N3:N4 is 4:2:5:4. The N 1s region for the nonirradiated sample was, then, fit with four components constrained to the referred atomic ratio. A good fit was obtained with components centered at 399.07, 399.20, 400.18, and 400.86 eV, values which are in good agreement with literature for $sp^2$ and $sp^3$ N 1s photoelectrons.[37,39] For the irradiated samples, the same set of peaks was imposed in what regards the binding energy differences but without any atomic ratio constraint. Fitted peaks were centered, for the set of samples analyzed, at 399.0 ± 0.1 eV (N1), 399.1 ± 0.1 eV (N2), 400.1 ± 0.1 eV (N3), and 400.8 ± 0.1 eV (N4).

Phosphorus P 2p region which, in all the samples, presented a single doublet with a spin orbit split of 0.9 eV and with the main component, P $2p_{3/2}$ centered at 133.4 ± 0.1 eV, is assignable to phosphorus in the phosphate group.[40]

Finally, Na 1s was fitted with a single peak centered at 1071.3 ± 0.3 eV. The fwhm was about 2.0 eV in all the samples, except for samples irradiated with 200 and 220 nm, where it shifted to 2.2 and 2.7 eV, respectively. This observation suggests that part of the $Na^+$ ions, instead of being next to the DNA strand, are water solvated. This leads to the broadening of the Na 1s peak as a result of neighborhood diversity increase.

Quantitative information may also be extracted from XPS data even in stratified samples such as LbL films, by taking into account the contribution to the total intensity of photoelectrons coming from different layers as performed in the study published in ref 41. Here, since no stratification is expected, the elemental composition values are presented as obtained without further geometrical considerations. They are listed in Table 1 for all the DNA samples together with the respective assignments.

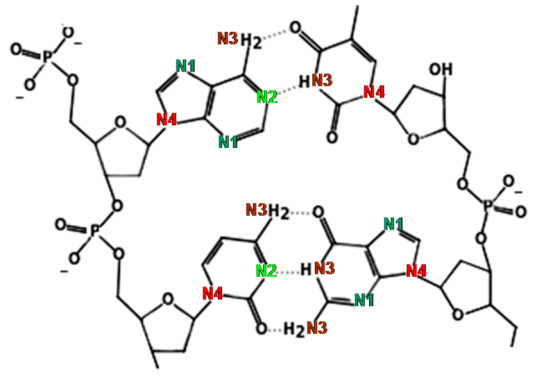


**Figure 3.** N 1s fitted components assignment.

**3.2. Damage Analysis.** Since XPS qualitative analysis shows that all the functional groups existing in the nonirradiated samples are kept in the irradiated ones, a quantitative analysis is required to follow the evolution of the relative amounts as a function of photon energy, using the values in Table 1. It should be remarked that part of the degradation products may be in the gaseous state at room temperature escaping the sample once they are produced. These products could be C, N, O, and/or P based. The only element which is not expected to escape from the sample is sodium, which was then used as the reference to evaluate the evolution of all the other elements with the UV energy increasing. All [X]/[Na] ratios are displayed in Figure 4 as a function of the radiation energy, where X is total P, C, O, and N. The UV absorption spectrum was superposed to try to establish a correlation between the promoted transition or ionization and the induced composition changes.

From Figure 4, two energy intervals may be identified where all the elements decrease their amounts with respect to sodium, strongly suggesting that dissociative degradation is taking place. Those intervals are ∼4.2 to ∼5.6 eV and ∼7 to 7.8 eV. It should be noted that, in the first interval, a strong UV absorption band is centered. Other authors found thresholds for dissociative DNA degradation, leading to the production of oxygen- and nitrogen-based fragments, between 3.5 and 5 eV, when irradiated with electron beams.[42] Here, it is clearly above 4.2 eV. Present data show also a decrease in phosphate groups concentration with UV irradiation; the [P]/[Na] ratio (Figure 4), in nonirradiated samples, is 1.4, indicating that a part of the phosphate groups are protonated, and consequently, DNA is behaving as a polyelectrolyte with an ionization degree around 0.7. Since sodium atoms are not leaving from DNA and the ratio between phosphorus and sodium is tending to unity as the radiation energy increases, this means that protonated phosphate groups are leaving from DNA. This conclusion is further supported by the presence of sodium counterions which can also be associated with DNA hydration[43] and is consistent with the observed presence of oxygen component associated with water (O4) and by the fact that water molecules start to absorb UV radiation at energies above 6.5 eV[44]. Therefore, a small interaction of radiation with the phosphate groups that are surrounded by water molecules and counterions is expected. This allows us to conclude that water molecules are playing a role of protecting the phosphate groups. For the highest value

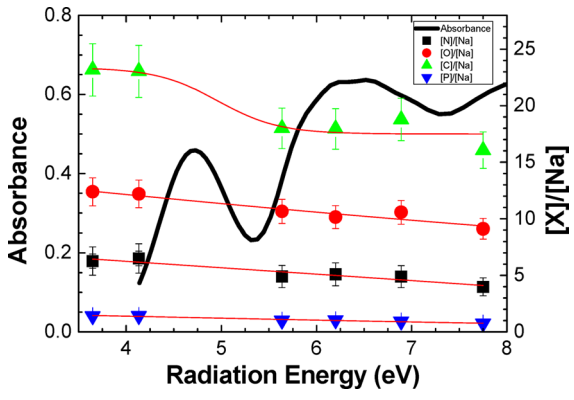


**Figure 4.** XPS atomic ratios between the X elements and sodium, where X is carbon, oxygen, nitrogen, and phosphorus. The UV absorption spectrum is superposed. The thin solid lines are guidelines.



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of irradiating energy, a [P]/[Na] ratio lower than 1 (0.75) is even reached. This means that other negatively ionized species are accumulating in the medium and that sodium becomes their counterion.

The detailed analysis of the evolution of the fitted components to both the XPS C 1s and O 1s regions as a function of the irradiation energy may also be useful. These plots are shown in Figure 5.

Plots of Figure 5a reveal that the components related to N−**C**═O, N−C═N, $C(N)_3$ and N−C−O (C3) and to N−(**C**═O)−N (C4) are the most robust under the action of UV radiation decreasing just slowly with the increasing radiation energy. The component related with C−O, C−N, and C═N (C2) has two clear decreasing steps in the energy ranges from 4.1 to 5.6 eV and from 6.9 to 7.7 eV. In the first energy interval, the anionic phosphate esters suffer the first ionization. The second one corresponds to the energy range where the intense UV absorption band is centered. The C−C or C−H (C1) component accompanies the decreasing of the C2 one in the first energy interval but, in the second energy interval, it increases while the C2 component decreases. This suggests that in the range from 4.1 to 5.6 eV, a dissociative damage is occurring, leading to the formation of gaseous products leaving the sample, affecting both the carbon bound to carbon and hydrogen and the carbon singly bound to oxygen. On the contrary, in the second interval, it seems that the damage consists of a transformation of a part of C2 into C1, compatible with an opening of C−O sugar bond.[12] The slow decrease of C4, since the group N−(C═O)−N exists only in thymine and cytosine, is related to the degradation of pyrimidines and does not exhibit a clear threshold in this energy range.

Analysis of graphs of Figure 5b reveal that the aromatic **O**═C (O1) has the same decreasing trend as C3 component, giving strength to the assignment presented in Table 1. The O−C (O3) relative amount keeps rather constant until ∼5.6 eV and clearly decreases for higher energies. This trend is indicating, once again, the rupture of the sugar ring, which may transform the C−O bond into a C═O one, explaining the behavior of the O1/Na ratio. Summarizing, quantitative analysis of XPS data related to carbon and oxygen elements indicates that radiation is affecting, mainly, sugar rings. However, since carbon and oxygen also exist in most common contaminants, oxygen is also bound to P, and its binding energy may vary with the phosphate degradation; the soundest elements to characterize samples degradation are nitrogen, phosphorus, and sodium. Thus, the effect of UV radiation on these elements will be discussed below.

The effect of UV radiation on DNA bases can be better analyzed by plotting, the atomic ratio between each of the N 1s components above-described (N1 to N4; see Figure 3 for the identification) and Na for the different DNA samples irradiated at different energies, as displayed in Figure 6. As a matter of fact, carbon and oxygen may exist also in contamination as well as in sugars and phosphate, respectively, while nitrogen is a good fingerprint of the DNA bases.

Plots of Figure 6 reveal that the large decrease of nitrogen occurs in the energy interval where also the anionic phosphate esters suffer the first ionization. However, the large concentration decrease, and specifically in that irradiating energy, affects mainly the components N1 and N4, that is, the nitrogen atoms which are not involved in hydrogen bonding. The N2 component has a very smooth variation, and N3 is practically unaffected except in the irradiating energy interval between 6.9 and 7.5 eV, where the intense UV absorption band is centered. Crossing this result with the previous observation that the C−O bond, just existing in sugars, was the most affected, decreasing in the same irradiating energy interval as

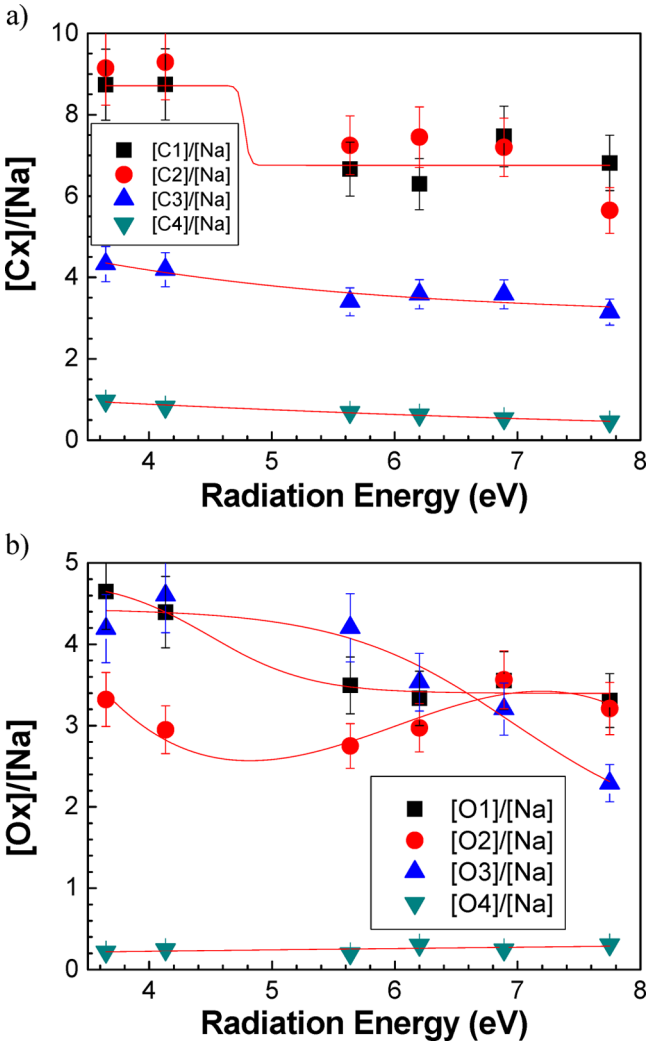


**Figure 5.** XPS atomic ratios between each (a) C 1s and (b) O 1s components and Na. The solid lines are guidelines.

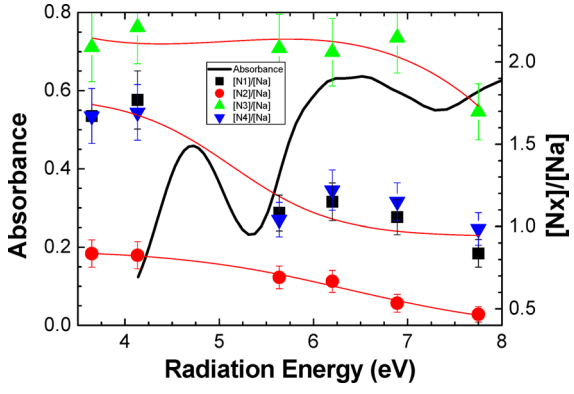


**Figure 6.** Atomic ratios [N*x*]/[Na] (with *x* = 1, 2, 3, and 4) as a function of the UV irradiating energy. The DNA absorbance spectrum was also included. The thin solid lines are guidelines.



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the N1 and N4 components, strongly suggests that the damage related with these depletions is caused by electrons that by attaching to species by an impact mechanism lead to dissociative states. Therefore, since the mean free electron path of these low-energy electrons is low, the main collision interactions occur with the nitrogen external groups in the ds-DNA. A similar result has already been obtained when DNA films were irradiated with an external electron beam with a range of very low-incident energy (0 to 10 eV).[39] The consequence of the interaction of these in situ generated very low kinetic energy photoelectrons is the protection of the most internal part of the double helix, which is formed of multiple hydrogen bonds, involving both N2 and N3 type (Figure 3) nitrogen atoms. The attachment of electrons with low energies, namely, those with energy below the ionization potential, initiate fragmentation of small molecules leading to the formation of a resonance, a transient molecular anion state, which decays by electron autodetachment or by dissociation along one, or several, specific bonds such as $RH^{*-} \rightarrow R' + H^-$.[7] Therefore, a selective loss of $H^-$ may occur.[21] However, the XPS results show that also nitrogen and oxygen are missing. It should be also mentioned that the Zheng et al.[45] studying the DNA damage by low-energy electrons concluded that electron most likely is initially captured by the base, forming a transient anion and, subsequently, the autodetaching electrons can be transferred to the sugar and phosphate group. Thus, the removal of phosphate groups by the photons reduce the number of electrons in the sugar groups, suggesting that electrons can be displaced from the bases to the sugar groups.

The effect of UV radiation is also critical on the phosphate groups themselves, as seen above in Figure 4 through the [P]/[Na] ratio. Also the stoichiometric ratio [C]/[P] may help to further clarify this issue. This ratio in DNA, neglecting end chain effects, should be [C]/[P] = 20/2 for the A-T pair and 19/2 for the G-C pair. Therefore, the expected ratio [C]/[P] should be between 9.5 and 10, depending on DNA base composition. The ratios of total carbon atomic percentages relative to phosphorus percentages increase with the radiation energy as deduced from Figure 4 plots, indicating that phosphate groups are being removed by the UV radiation more extensively than carbon. In the irradiating energy range investigated, phosphorus decreases with respect to all the elements, suggesting that the threshold for damaging phosphate groups is 4.2 eV or lower. However, since the DNA conductivity decreases with the loss of phosphates as demonstrated in ref 18 and different conductivity values are found in literature for DNA samples[46] then this suggests that the damage of the phosphate group has a lower threshold.

By plotting the atomic ratios [P]/[Na] and [N$x$]/[Na] (with $x$ = 1 and 4) as a function of the UV irradiating energy, it can be observed that all of these ratios follow the same trend (see Figure 7). This allows us to conclude that damage induced on DNA bases, namely the losses of N1 and N4, is directly related with the losses of phosphate groups. One explanation for these results can be translated by the following mechanism: during the phosphate group's removal, low-energy electrons can be ejected from the DNA bases due to formation of resonances, consequently leading to the loss of N1 and N4, as described above.

The values of energy required to damage the different DNA components discussed are summarized in Table 2. The agreement of values of energy when different elements for the same chemical group are analyzed demonstrates the reliability of the obtained values.

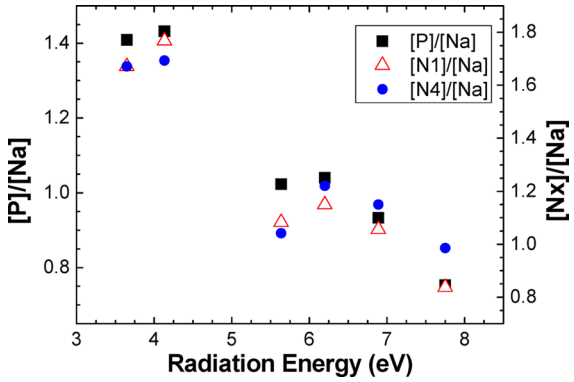


**Figure 7.** Atomic ratios [P]/[Na] and [N$x$]/[Na] (with $x$ = 1 and 4) as a function of the UV irradiating energy.

## 4. CONCLUSIONS

Synchrotron UV radiation in the energy range of 3.5 to 8 eV was shown to cause damage to several DNA components as revealed by XPS spectra analysis of DNA cast films. Analysis of XPS atomic ratios between all the elements (X) and sodium reveals two main energy intervals where the relative amount of X decreases, from ~4.2 to ~5.6 eV and from ~7 to 7.8 eV, strongly suggesting that the absorption of UV leads to dissociative damage of DNA. A detailed study of the evolution of each component relative amount with radiation energy reveals some group transformations. From 4.1 to 5.6 eV, the decrease of ratio between the C 1s component assigned to C−O, C−N, and C═N (C2), and sodium, shows that there is a dissociative damage, leading to the formation of gaseous products N- and/or O-containing. However, from 6.9 to 7.7 eV, part of this C2 carbon transforms into aliphatic C−C and C−H, compatible with an opening of C−O sugar bond, as confirmed by the (**O**−C) from O 1s/Na ratio decrease for higher energies. Also, the C−O bond may transform into a C═O as inferred from the (C═**O**) from O 1s/Na ratio.

Interestingly, a large decrease of the nitrogen atoms not involved in hydrogen bonding occurs for lower UV radiation energies, specifically for energies where also the anionic phosphate esters suffer the first ionization, while nitrogen atoms involved in hydrogen bonds vary rather smoothly with UV energy. The only exception is the amine nitrogen atoms acting as electron acceptors, which markedly decrease for

**Table 2. Summary of the Energy Values (±0.1 eV) of the Different DNA Groups Damaged**

| DNA group component | energy (eV) | XPS components |
|---|---|---|
| phosphate | 4.2 eV or lower | (O2 + O3) |
| | | P |
| DNA bases | >4.2 | N $sp^2$ (N1) and N $sp^3$ (N4) not involved in hydrogen bonding |
| | >6.9 | acceptor N $sp^3$ involved in hydrogen bonding (N3) |
| sugar ring (deoxyribose) | >6.9 | C2 (C−O) |
| | | O3 (O−C) |
| | | O1 (C═O) |



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energies larger than 6.9 eV, coinciding with the intense UV absorption band. These results strongly suggest that nitrogens near the central axis of the ds-DNA helix are far more shielded from damage than the peripheral nitrogen atoms in the DNA bases. This result is compatible with a damage mechanism not directly promoted by the UV photons but rather by the photoelectrons ejected by the bases since phosphate ester groups are the species with lower ionization potential in the system. These photoelectrons with very low energy came from the most peripheral nitrogen groups near sugars due to the formation of transient molecular anion states which decay by electron autodetachment. Analysis of the XPS data, associated with phosphate groups, pointed out that an energy below 3.5 eV is sufficient to break DNA phosphate groups, a fact which is useful in accounting for the different conductivity values found in literature for DNA samples and can explain the ejection of a very low-energy electron during the phosphate group removal. This electron can migrate through the DNA bases, causing a resonance formation and DNA base fragmentation.

Finally, analysis of XPS [P]/[Na] ratio which changed from ∼1.4 to ∼0.75 as radiation energy increases, revealed mainly that the protonated phosphate groups are being removed and not the ionized ones. This allows one to conclude that ionized phosphate groups, surrounded by the sodium counterions, congregate hydration water molecules which play a UV radiation protection role.

## AUTHOR INFORMATION


### Corresponding Authors

*E-mail: ana.ferraria@tecnico.ulisboa.pt. Tel: 00351 218419258/7.

*E-mail: mfr@fct.unl.pt. Tel: 00351 212948576.


### Notes

The authors declare no competing financial interest.

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## REFERENCES


(1) Hanel, G.; Gstir, B.; Denfil, S.; Scheier, P.; Probst, M.; Farizon, B.; Farizon, M.; Illenberger, E.; Mark, T. D. Electron attachment to uracil: Effective destruction at subexcitation energies. *Phys. Rev. Lett.* **2003**, *90*, 188104.

(2) Alizadeh, E.; Sanz, A. G.; Garcia, G.; Sanche, L. Radiation Damage to DNA: The Indirect Effect of Low-Energy Electrons. *J. Phys. Chem. Lett.* **2013**, *4* (5), 820−825.

(3) Pfeifer, G. P.; You, Y.-H.; Besaratinia, A. Mutations induced by ultraviolet light. *Mutat. Res.* **2005**, *571*, 19−31.

(4) Cadet, J.; Sage, E.; Douki, T. Ultraviolet radiation-mediated damage to cellular DNA. *Mutat. Res.* **2005**, *571*, 3−17.

(5) Pfeifer, G. P. How the environment shapes cancer genomes. *Curr. Opin. Oncol.* **2015**, *27* (1), 71−77.

(6) Yokoyama, H.; Mizutani, R. Structural Biology of DNA (6−4) Photoproducts Formed by Ultraviolet Radiation and Interactions with Their Binding Proteins. *Int. J. Mol. Sci.* **2014**, *15* (11), 20321−20338.

(7) Boudaïffa, B.; Cloutier, P.; Hunting, D.; Huels, M. A.; Sanche, L. Resonant Formation of DNA Strand Breaks by Low-Energy (3 to 20 eV) Electrons. *Science* **2000**, *287*, 1658−1660.

(8) Berdys, J.; Anusiewicz, I.; Skurski, P.; Simons, J. Damage to model DNA fragments from very low-energy (< 1 eV) electrons. *J. Am. Chem. Soc.* **2004**, *126*, 6441−6447.

(9) Folkard, M.; Prise, K. M.; Brocklehurst, B.; Michael, B. D. DNA damage induction in dry and hydrated DNA by synchrotron radiation. *J. Phys. B: At., Mol. Opt. Phys.* **1999**, *32*, 2753−2761.

(10) Folkard, M.; Prise, K. M. Investigating mechanisms of radiation-induced DNA damage using low-energy photons. *Acta Phys. Pol., A* **2006**, *109*, 265−271.

(11) Huels, M. A.; Boudaiffa, B.; Cloutier, P.; Hunting, D.; Sanche, L. Single, Double, and Multiple Double Strand Breaks Induced in DNA by 3−100 eV Electrons. *J. Am. Chem. Soc.* **2003**, *125*, 4467−4477.

(12) Sabin, J. R. Theory of the interaction of radiation with biomolecules; Advances in Quantum Chemistry; Elsevier/Academic Press: Amsterdam, The Netherlands, 2007; Vol. *52*.

(13) Gomes, P. J.; Ribeiro, P. A.; Shaw, D.; Mason, N. J.; Raposo, M. UV Degradation of Deoxyribonucleic Acid. *Polym. Degrad. Stab.* **2009**, *94*, 2134−2141.

(14) Almeida, D.; da Silva, F. F.; García, G.; Limão-Vieira, P. Selective bond cleavage in potassium collisions with pyrimidine bases of DNA. *Phys. Rev. Lett.* **2013**, *110*, 023201.

(15) Baccarellia, I.; Baldc, I.; Gianturco, F. A.; Illenberger, E.; Kopyrad, J. Electron-induced damage of DNA and its components: Experiments and theoretical models. *Phys. Rep.* **2011**, *508*, 1−44.

(16) Arumainayagam, C. R.; Lee, H.-L.; Nelson, R. B.; Haines, D. R.; Gunawardane, R. P. Low-energy electron-induced reactions in condensed matter. *Surf. Sci. Rep.* **2010**, *65*, 1−44.

(17) Zheng, Yi; Sanche, L. Low energy electrons in nanoscale radiation physics: Relationship to radiosensitization and chemo-radiation therapy. *Reviews in Nanoscience and Nanotechnology* **2013**, *2*, 1−28.

(18) Gomes, P. J.; Coelho, M.; Dionısio, M.; Ribeiro, P. A.; Raposo, M. Probing Radiation Damage by Alternated Current Conductivity as a Method to Characterize Electron Hopping Conduction in DNA Molecules. *Appl. Phys. Lett.* **2012**, *101*, 123702.

(19) Rabitz, H.; de Vivie-Riedle, R.; Motzkus, M.; Kompa, K. Whither the Future of Controlling Quantum Phenomena? *Science* **2000**, *288*, 824−828.

(20) Wada, S.; Sumii, R.; Isari, K.; Waki, S.; Salo, E. O.; Sekiguchi, T.; Sekitani, T.; Tanaka, K. Active Control of Chemical Bond Scission by Site-Specific Core Excitation. *Surf. Sci.* **2003**, *528*, 242−248.

(21) Ptasinska, S.; Denifl, S.; Grill, V.; Märk, T. D.; Illenberger, E.; Scheier, P. Bond- and Site-Selective Loss of $H^-$ from Pyrimidine Bases. *Phys. Rev. Lett.* **2005**, *95*, 093201.

(22) Prabhudesai, V. S.; Kelkar, A. H.; Nandi, D.; Krishnakumar, E. Functional group dependent site specific fragmentation of molecules by low energy electrons. *Phys. Rev. Lett.* **2005**, *95*, 143202.

(23) Sloan, P. A.; Palmer, R. E. Two-electron dissociation of single molecules by atomic manipulation at room temperature. *Nature* **2005**, *434*, 367−371.

(24) Bald, I.; Kopyra, J.; Illenberger, E. Selective excision of C5 from D-ribose in the gas phase by low-energy electrons (0−1 eV): Implications for the mechanism of DNA damage. *Angew. Chem., Int. Ed.* **2006**, *45*, 4851−4855.

(25) Fielding, H. H.; Robb, M. A. Quantum molecular dynamics and control. *Phys. Chem. Chem. Phys.* **2010**, *12*, 15569−15569.

(26) Ratschbacher, L.; Zipkes, C.; Sias, C.; Köhl, M. Controlling chemical reactions of a single particle. *Nat. Phys.* **2012**, *8*, 649.

(27) So, R.; Alavi, S. Vertical excitation energies for ribose and deoxyribose nucleosides. *J. Comput. Chem.* **2007**, *28*, 1776−1782.

(28) Fetzer, S. M.; Lebreton, P. R.; Rohmer, M.-M.; Veillard, A. Valence ionization potentials of anionic phosphate esters: An ab initio quantum mechanical study. *Int. J. Quantum Chem.* **1997**, *65*, 1095−1106.




DOI: 10.1021/acs.jpcb.5b01439
*J. Phys. Chem. B* 2015, 119, 5404−5411

(29) Shukla, M. K.; Leszczynski, J. TDDFT Investigation on Nucleic Acid Bases: Comparison with Experiments and Standard Approach. *J. Comput. Chem.* **2004**, *25*, 768−778.
(30) Borin, A. C.; Serrano-Andrés, L.; Fülscher, M. P.; Roos, B. O. A Theoretical Study of the Electronic Spectra of $N_9$ and $N_7$ Purine Tautomers. *J. Phys. Chem. A* **1999**, *103*, 1838−1845.
(31) Brondsted-Nielsen, S.; Chakraborty, T.; Hoffmann, S. V. Synchrotron Radiation Circular Dichroism Spectroscopy of Ribose and Deoxyribose Sugars, Adenosine, AMP and dAMP Nucleotides. *ChemPhysChem* **2005**, *6*, 2619−2624.
(32) Roca-Sanjuán, D.; Rubio, M.; Merchán, M.; Serrano-Andrés, L. *Ab initio* determination of the ionization potentials of DNA and RNA nucleobases. *J. Chem. Phys.* **2006**, *125*, 084302.
(33) Eden, S.; Limao-Vieira, P.; Hoffmann, S. V.; Mason, N. J. VUV photoabsorption in CF(3)X (X = Cl, Br, I) fluoro-alkanes. *Chem. Phys. Lett.* **2006**, *323*, 313−333.
(34) Ferreira, Q.; Gomes, P. J.; Ribeiro, P. A.; Jones, N. C.; Hoffmann, S. V.; Mason, N. J.; Oliveira, O. N., Jr.; Raposo, M. Determination of degree of ionization of poly(allylamine hydrochloride) (PAH) and poly[1-[4-(3-carboxy-4 hydroxyphenylazo)-benzene sulfonamido]-1,2-ethanediyl, sodium salt] (PAZO) in layer-by-layer films using vacuum photoabsorption spectroscopy. *Langmuir* **2013**, *29*, 448−455.
(35) Botelho do Rego, A. M.; Ferreira, L. F. V. *In Handbook of Surfaces and Interfaces of Materials*; Nalwa, H. S., Ed.; Academic Press: New York, 2001; Vol. 2, pp 243−313.
(36) *XPSPeak 4.1*, freeware (www.xpspeak.software.informer.com/4.1/).
(37) Beamson, G.; Briggs, D. High Resolution XPS of Organic Polymers. *The Scienta ESCA300 Database*; John Wiley & Sons: New York, 1992.
(38) May, C. J.; Canavan, H. E.; Castner, D. Quantitative X-ray Photoelectron Spectroscopy and Time-of-Flight Secondary Ion Mass Spectrometry Characterization of the Components in DNA. *Anal. Chem.* **2004**, *76*, 1114−1122.
(39) Rei Vilar, M.; Botelho do Rego, A. M.; Ferraria, A. M.; Jugnet, Y.; Noguès, C.; Peled, D.; Naaman, R. Interaction of self-assembled monolayers of DNA with electrons: HREEL and XPS studies. *J. Phys. Chem. B* **2008**, *112*, 6957−6964.
(40) Naumkin, A. V.; Kraut-Vass, A.; Gaarenstroom, S. W.; Powell, C. J. NIST X-ray Photoelectron Spectroscopy Database, NIST Standard Reference Database 20, version 4.1, http://srdata.nist.gov/xps/ (accessed May 2014).
(41) Raposo, M.; Lourenco, J. M. C.; Botelho do Rego, A. M.; Ferraria, A. M.; Ribeiro, P. A. Counterions: A new approach to control the degree of ionization of polyelectrolytes in layer-by-layer films. *Colloids Surf., A* **2012**, *412*, 1−10.
(42) Boulanouar, O.; Fromm, M.; Mavon, C.; Cloutier, P.; Sanche, L. Dissociative electron attachment to DNA-diamine thin films: Impact of the DNA close environment on the OH and O decay channels. *J. Chem. Phys.* **2013**, *139*, 055101.
(43) Savelyev, A.; Papoian, G. A. Electrostatic, Steric, and Hydration Interactions Favor $Na^+$ Condensation around DNA Compared with $K^+$. *J. Am. Chem. Soc.* **2006**, *128*, 14506−14518.
(44) Mota, R.; Parafita, R.; Giuliani, A.; Hubin-Franskin, M. J.; Lourenco, J. M. C.; Garcia, G.; Hoffmann, S. V.; Mason, N. J.; Ribeiro, P. A.; Raposo, M.; Limao-Vieira, P. Water VUV Electronic State Spectroscopy by Synchrotron Radiation. *Chem. Phys. Lett.* **2005**, *416*, 152−159.
(45) Zheng, Y.; Richard Wagner, J.; Sanche, L. DNA Damage Induced by Low-Energy Electrons: Electron Transfer and Diffraction. *Phys. Rev. Lett.* **2006**, *96*, 208101.
(46) Endres, R. G.; Cox, D. L.; Singh, R. R. P. *Colloquium*: The quest for high-conductance DNA. *Rev. Mod. Phys.* **2004**, *76*, 195−214.


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