Influence of a charged graphene surface on the orientation and conformation of covalently attached oligonucleotides: a molecular dynamics study†

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Molecular dynamics (MD) simulations of single-stranded (ss) and double-stranded (ds) oligonucleotides anchored via an aliphatic linker to a graphene surface were performed in order to investigate the role of the surface charge density in the structure and orientation of attached DNA. Two types of interactions of DNA with the surface are crucial for the stabilisation of the DNA–surface system. Whereas for a surface with a zero or low positive charge density the dispersion forces between the base(s) and the surface dominate, the higher charge densities applied on the surface lead to a strong electrostatic interaction between the phosphate groups of DNA, the surface and the ions. At high-charge densities, the interaction of the DNA with the surface is strongly affected by the formation of a low-mobility layer of counterions compensating for the charge of the surface. A considerable difference in the behaviour of the ds-DNA and ss-DNA anchored to the layer was observed. The ds-DNA interacts with the surface at low- and zero-charge densities exclusively by the nearest base pair. It keeps its geometry close to the canonical B-DNA form, even at surfaces with high-charge densities. The ss-DNA, owing to its much higher flexibility, has a tendency to maximise the attraction to the surface exploiting more bases for the interaction. The interaction of the polar amino group(s) of the base(s) of ss-DNA with a negatively charged surface also contributes significantly to the system stability.

I. Introduction

Microarrays or DNA chips are arrays of tens to tens of thousands of microscopic spots containing single-stranded deoxyribonucleotides attached to a solid surface (such as a membrane, a polymer, or glass) used to analyse simultaneously a sample solution containing fragments of nucleic acids. Oligonucleotides (capture probes) in individual spots are identical, but their sequences are different for each spot to match the various complementary DNA sequences (targets) present in a given sample. The extent of binding of a complementary fragment to the surface-attached oligonucleotides is detected mainly fluorometrically.1–5

Originally, the main application of microarrays was gene-expression screening. At present, microarrays can be used to detect DNA or RNA sequences of pathogens, organisms, mutations or generally speaking any characteristic sequence of any object of interest: DNA microarrays are used for the detection and identification of bacteria and genes of interest from various environments (e.g. soil, sediment, water column),6 they are suitable for the detection of single nucleotide polymorphisms7,8 and for many other practical applications, such as for example the detection of viruses.9–11

The sequences of surface-immobilised capture probes are designed in a similar manner and according to the same principle as those for probes or primers in a bulk solution: they should not allow the formation of internal structures such as hairpins. They should be sensitive to sequence variations and bind only to complementary strands. The hybridisation on an array requires a similar melting temperature for all of the capture probes. There are many software tools available for probe design that are used during the process of microarray development such as those presented by Li and Wernersson.12,13 Nevertheless, all of them are based on standard hybridisation conditions, i.e. nucleic acids in solution, not being attached to a surface.

However, the surface plays an important role. First, the capture probes are immobilised to a certain extent by surface binding and therefore their molecular dynamics is different
from that describing a system of two free strands in a solution. Second, the presence of the surface and the interactions of the capture probe with the surface represent an important steric hindrance making single-strand–double-strand transitions more difficult. Third, in addition to the van der Waals interactions, the surface, whether it is charged or not, generates nontrivial electrostatics and interfacial structure owing to the interactions between the solution and the surface, influencing the density profiles of the water and ions in the vicinity of the surface and attached probes.

In order to accelerate hybridisation, various experimental enhancements have been proposed, such as flow-chamber hybridisation and the application of electrostatic fields. The first steps to estimate some of the aspects concerning the influence of the surface have already been made, based both on experiments\textsuperscript{14,15} and on theoretical considerations\textsuperscript{16,17} but the underlying phenomena remain poorly understood.

This molecular study elucidates the role of the surface and the electric field in the spatial distribution of ions, the behaviour of the probes and targets and in the hybridisation process. The anchored microarrays on the surface are represented by a single-stranded oligomer of DNA, whereas the hybridised ones are represented by a double-stranded DNA with one strand (probe) covalently attached to the surface.

Various surfaces are used in biosensing and micro-array technologies. The most used one by far is a silica surface usually covered with various organic molecules in order to enhance or modify its hybridisation efficiency.\textsuperscript{18–20} Other types of surfaces include various materials such as polymers, poly(methyl-meta acrylate) (PMMA),\textsuperscript{21,22} poly(dimethylsiloxane) (PDMS),\textsuperscript{23} polypropylene (PP),\textsuperscript{24} gold,\textsuperscript{25,26} graphene,\textsuperscript{27–30} etc. The graphene surface has been selected as the substrate in our simulations, because it is a relatively simple but realistic surface and its structure is independent of pH, salt concentration, etc. Finally, some theoretical studies on DNA interaction with carbon nanotubes (with surfaces based on graphene geometry) have already been conducted.\textsuperscript{31–37} The binding of DNA to carbon nanotubes has been identified as a way to open the door to carbon-nanotube-based applications in biotechnology.\textsuperscript{34}

II. Simulation methods

A. System setup

Two graphene layers of a size of 68.16 $\times$ 66.41 Å and a separation of 61.40 Å were generated by the BuildCstruct 1.1 program.\textsuperscript{38} Each layer consists of 1728 carbon atoms. The rectangular box contains the system of interest, i.e. DNA attached to a charged surface, water and ions. The role of the second graphene surface, which was always neutral, was to close the system. In order to minimise the influence of the electric field generated by the charged graphene layer on the periodic images, the replicas of the system were separated by a 100 Å vacuum gap. The whole model is depicted in Fig. 1.

The initial structures of both the double-stranded (ds-DNA) and single-stranded decamers (ss-DNA) with the base sequence 5'–CCACTAGTGG–3' in the canonical B-form were generated using the NAB module implemented in the AmberTools 1.2 package.\textsuperscript{39}

The oligonucleotide was covalently bonded to the graphene layer via an aliphatic carbon linker consisting of six methylene groups (C6). The linker was attached to the 5' end of the cytosine nucleotide by the phosphodiester bond. In the initial position, the DNA helix axis was orientated perpendicularly to the graphene slab.

In order to test the role of the charge of the graphene layer in the position and the orientation of the DNA, all of the carbon atoms of the graphene layer, to which DNA is anchored, were charged to one of the following values: $-0.1$, $-0.05$, $-0.02$, $-0.01$, $0$, $+0.01$, $+0.02$, $+0.05$ or $+0.1$ elementary charge ($e$) per carbon, corresponding to the range of the charge densities $-0.594$ to $+0.594$ C m\textsuperscript{-2} comparable to those used in the experiments.\textsuperscript{40,41} The sodium/chloride ions were used to neutralise fully the charge applied on the graphene, whereas the DNA was neutralised independently by sodium cations. All of the nominal charges per carbon atom, the charge densities and the number of ions used in the simulations are summarised in Table 1.

The probe surface density used in our study ($2 \times 10^{12}$ cm\textsuperscript{-2}) was selected to be of the same order of magnitude as a microarray maximum sensitivity for a 10-mer long probe ($6 \times 10^{12}$ cm\textsuperscript{-2}).\textsuperscript{42}

For comparison, ds-DNA and ss-DNA with a minimum distance between the solute and boundaries set to 10 Å in a rectangular periodic box with no graphene layers were also simulated.

B. Parameters

The parmbsc0\textsuperscript{43} modification of the parm99 force field for DNA was used, together with the TIP3P\textsuperscript{44} water model and Na\textsuperscript{+} counterions neutralising the negative charge of the DNA. New parameters\textsuperscript{45} for Na\textsuperscript{+} and Cl\textsuperscript{-} ions were adopted to prevent their cocrystallisation. The 'CA' atomic type for the
The DNA conformation was described using the parameters or step parameters (tilt, roll, twist, shift, slide and rise) were prolonged to 100 ns. The structures were saved every 1 ps.

The electric charge axial densities were analysed by the g potential utility of the GROMACS software package.  

### III. Results and discussion

#### A. Double stranded DNA

The deviation of the helical axis from the normal vector of the graphene plane depending on the charge of the graphene layer is shown in Fig. 2. The distance of the phosphorus atom of ds-DNA, localised in the middle of the backbone of the closer strand of DNA to the layer, from the charged graphene layer during the MD simulation is shown in Fig. 3 for all of the studied atomic charges of the graphene atoms. The averaged values of above-mentioned properties from the already equilibrated part of the trajectory (last 70 ns) can be found in Table 2. The typical snapshots from the MD trajectories are depicted in Fig. 4.

The free ds-DNA oligomer shows local features consistent with the B-DNA conformational family. Apart from the terminal base pairs and steps, the oligomer retains its B-DNA-like conformation also in the presence of graphene, regardless of the charge of the graphene layer. See Table S1 (ESI†) for a list of the average local parameters.

**Uncharged graphene.** The interaction between the DNA and uncharged graphene is dominantly governed by dispersion forces mediated by the closest base pair orientated parallel with the graphene layer (Fig. 4). After approximately 5 ns of the MD simulation, the DNA is attracted to the graphene slab by changing the originally extended conformation of the linker to the coiled one. This is characterised by a shortening of the original distance of the central phosphorus atom of DNA to the graphene from the initial 27 Å to ~15 Å. No further changes in the orientation of the DNA were observed for the rest of the simulation. The DNA remains more or less perpendicularly orientated to the graphene for the whole time of the simulation (an average deviation from perpendicularity of ~18 degrees; see Table 2). Zhao  found similar results for a self-assembled layer of four ds-DNAs on the graphene surface. The angle between the DNA axes and the graphene-normal vector in his study varied between 0 and 40 degrees with an average of around 25 degrees. The dispersion interaction

<table>
<thead>
<tr>
<th>Nominal charge (e/carbon)</th>
<th>Real charge (e/carbon)</th>
<th>Charge density (C m⁻³)</th>
<th>Number of ions</th>
<th>ds-DNA</th>
<th>ss-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>−0.10</td>
<td>−0.09954</td>
<td>−0.594</td>
<td>191</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>−0.05</td>
<td>−0.04977</td>
<td>−0.297</td>
<td>105</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>−0.02</td>
<td>−0.01967</td>
<td>−0.118</td>
<td>53</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>−0.01</td>
<td>−0.00984</td>
<td>−0.059</td>
<td>36</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.00</td>
<td>0.00000</td>
<td>0.000</td>
<td>19</td>
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<td>0.01</td>
<td>0.00984</td>
<td>0.059</td>
<td>19</td>
<td>17</td>
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<tr>
<td>0.02</td>
<td>0.01967</td>
<td>0.118</td>
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<td>0.04977</td>
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</tr>
<tr>
<td>0.10</td>
<td>0.09954</td>
<td>0.594</td>
<td>19</td>
<td>172</td>
<td>172</td>
</tr>
</tbody>
</table>

**Table 1** Nominal and real charges per carbon atom, charge densities and number of ions used in the simulations

Carbon atoms of graphene were employed similarly as in other papers simulating carbon-nanotube–DNA interactions. The partial charges and structural parameters of the aliphatic C6 linker were found using the standard RESP procedure.
between a carbon nanotube (CNT) and the closest base pair of ds-DNA was also observed in the work of Zhao and Johnson. 46

Negatively charged graphene. Already a relatively very small charge on each carbon atom of graphene (−0.01e) leads to qualitatively different results in comparison with the system containing uncharged graphene. The negatively charged phosphate groups of DNA are repelled by the negatively charged graphene layer, which results in the preservation of the extended conformation of the linker. The average distance of the central phosphorus of the DNA to the graphene is around 21 Å, but it fluctuates between 15 and 35 Å. The DNA orientation to the graphene surface is quite variable, between 0 and 70 degrees, with the average value of 42 degrees.

Similar conclusions can be drawn about the system containing graphene with an atomic charge of −0.02e. At the time of 11 ns, the disruption of H-bonds in the base pair closest to the graphene was observed. The cytosine base became orientated perpendicularly to the plane of the other bases starting to interact with the negatively charged slab via its amino group with partial positive charge. This motif is unique among all of the other systems of ds-DNA with negatively charged graphene, but it is frequently observed for systems containing ss-DNA. This behaviour of the ds-DNA can be explained by a destabilisation role of a negatively charged surface in the stability of the DNA duplex.51

When the charge of the carbon atoms of the graphene layer is enhanced to −0.05e, no new significant qualitative differences are observed in comparison with the less charged system. However, a slightly stronger preference of DNA to be situated closer to this surface than to the surface with charge −0.02 was detected, especially in the second half of the simulation. This behaviour can be explained by a significantly higher amount of sodium counterions situated close to the negatively charged graphene layer and having smaller mobility in comparison with simulations of less charged graphene. Thus, the negative phosphate groups of DNA start to feel the presence of the ordered counterions’ layer. This can be deduced from the decrease of the average distance of the central phosphorus atom to the slab from 25 Å to 14 Å. Zhao 46 conducted simulations of unattached ds-DNA interacting with the negatively charged CNT (−0.05e per atom). As expected, since there was no linker, the DNA segment rapidly moved away from the CNT.

Applying the charge of −0.1e to all of the carbons of the graphene leads to rather surprising results. The surface of

Fig. 2 The deviation of the helical axis of ds-DNA from the normal vector of the graphene plane in dependence on the charge of the graphene layer. The charge of the graphene atoms is indicated in each plot. Zero angle denotes the perpendicular orientation to the surface.
the graphene is fully covered by the layer of sodium cations with very low mobility. Thus, already in the equilibration period the DNA molecule is attracted to the graphene–sodium ‘bilayer’. The direct contacts with cations are mostly mediated by phosphates, situated at the edges of the DNA, whereas the central part of DNA is somewhat bent, as characterised by the average value of the deviation of the helical axis from the normal vector of the graphene around 50 degrees. The average distance of the central phosphorus atom to the graphene remains constant at about 5 Å during almost all of the time of the simulation.

**Positively charged graphene.** The smallest positive charge applied to carbon atoms (+0.01e) has a rather minor influence on the distance and orientation of the DNA in comparison with the uncharged graphene. The dispersion forces still contribute dominantly to the attraction of DNA to the slab. However, a bend of the upper part of DNA (i.e. the part more distant from the slab) leading to an approach of this part to the layer indicates that the electrostatic attraction between the negatively charged phosphates of DNA and the positively charged slab is already non-negligible.

An increase of the charge of the graphene to +0.02e leads to mutual cooperation of the electrostatic and dispersion interactions between the DNA and the substrate. The closest base pair to the surface still interacts with the surface by dispersion forces, while the rest of the DNA is bent and orientated closer to the surface, allowing thus the interaction of the negative phosphates of the nearer strand with the positive graphene layer. It leads to the decrease of the distance of the central phosphorus atom of DNA to the slab from 15 Å to 10 Å in comparison with the previous simulation.

The averaged properties (for the definition see the Methods section) characterizing the orientation of the DNA to the graphene layer

<table>
<thead>
<tr>
<th>Charge (e/carbon)</th>
<th>Double stranded DNA</th>
<th>Single stranded DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distance/Å</td>
<td>Angle/°</td>
</tr>
<tr>
<td>−0.10</td>
<td>4.9 ± 0.4</td>
<td>49.4 ± 4.8</td>
</tr>
<tr>
<td>−0.05</td>
<td>14.7 ± 4.9</td>
<td>47.4 ± 17.7</td>
</tr>
<tr>
<td>−0.02</td>
<td>24.2 ± 2.0</td>
<td>53.9 ± 9.6</td>
</tr>
<tr>
<td>−0.01</td>
<td>21.6 ± 3.3</td>
<td>42.0 ± 13.3</td>
</tr>
<tr>
<td>0.00</td>
<td>15.0 ± 2.1</td>
<td>18.0 ± 6.9</td>
</tr>
<tr>
<td>+0.01</td>
<td>14.0 ± 2.4</td>
<td>29.1 ± 8.4</td>
</tr>
<tr>
<td>+0.02</td>
<td>10.0 ± 0.9</td>
<td>53.9 ± 3.6</td>
</tr>
<tr>
<td>+0.05</td>
<td>10.4 ± 0.6</td>
<td>80.2 ± 4.3</td>
</tr>
<tr>
<td>+0.10</td>
<td>5.6 ± 0.2</td>
<td>79.4 ± 4.7</td>
</tr>
</tbody>
</table>

**Fig. 3** The distance of the central phosphorus atom of ds-DNA from the graphene layer during the MD simulation. The charge of the graphene atoms is indicated in each plot.
When the charge is increased to $+0.05e$, the electrostatic attraction becomes fully dominant, the ds-DNA is oriented parallel to the surface and thus no stacking interaction between the bases and the surface is observed.

However, since the canonical B-DNA structure of the ds-DNA is more or less conserved during the simulations, not all the phosphates interact with the surface. The middle part of the DNA remains somewhat deflected upon the graphene layer. This is probably the reason that the distance of the central phosphorus atom remains similar to the one in the case of the simulation with a less charged ($+0.02e$) graphene. The results agree well with the conclusions of Zhao and Johnson, who simulated free ds-DNA adsorbed at the positively charged CNT ($+0.05e$ per atom). They observed a similar bent structure of DNA with the ends closely attached to the CNT and slightly desorbed in the middle part of the DNA. This comparison might indicate the key role of stacking and electrostatic interactions of ds-DNA with carbon layers generally, regardless of the changes in the curvature of the surface.

For the system with the graphene charge of $+0.1e$, like in the case of graphene with carbons with a charge of $-0.1e$, the surface is fully covered by an almost immobile layer of counterions (here $\text{Cl}^-$). Initially, a rather strong repulsion between the chloride anions and phosphates leads to the extension of the linker as well as the DNA, resulting in a distance of the central phosphorus atom from the surface of about 35 Å. However, in the next 15 ns, the DNA bent down to the surface and eventually succeeded to penetrate through the layer of $\text{Cl}^-$ to the graphene surface. It started to interact with the positive layer of graphene via the phosphates situated in the central part of DNA as well as at the terminal part of the DNA opposite the linker. The phosphates at the closest part of the DNA to the linker are far from the surface and thus do not contribute to the stabilisation of the DNA–graphene complex. This situation remains unchanged until the end of the simulation.

B. Single stranded DNA

The deviation of the helical axis of the ss-DNA from the normal vector of the graphene plane is shown in Fig. 5. The distance of the central phosphorus atom of the ss-DNA to the layer is provided in Fig. 6. The averaged values of above-mentioned properties from the already equilibrated part of the trajectory (last 70 ns, resp. 60 ns for the system with graphene of charge $+0.1$) can be found in Table 2. The typical snapshots from the MD trajectories are depicted in Fig. 7.

The free ss-DNA oligomer exhibits a regular, stacked structure. Neglecting the end bases, the inter-base parameters are not far from their double-stranded counterparts, with the notable exceptions of high positive shift and high twist. However, if a graphene layer is present, the ss-DNA shows severe structural distortions. This applies to all of the charges of the graphene layer, including the zero charge. A visual inspection of the MD snapshots suggests that the ss-DNA, being much more flexible than ds-DNA, uses its conformational freedom to optimise contacts with the graphene layer. The average local conformational parameters are given in Table S2 (ESI†).

Uncharged graphene. The first few nanoseconds of the simulation of the interaction of the ss-DNA with the uncharged graphene provide a picture similar to the simulation of ds-DNA,
i.e. the interaction with the surface is mediated only by the closest base to the surface.

However, an enormous flexibility of the sugar-phosphate backbone later led to a sorption of the unattached terminal base to the graphene surface and after several nanoseconds to a spreading of the single-stranded oligonucleotide onto the surface with some bases stacked and some not. Our conclusions are in agreement with other theoretical and experimental publications: Shi et al. studied the adhesion and peeling of ss-DNA from an uncharged graphite surface and observed the DNA bases situated parallel to the surface with not all of the bases simultaneously attached to the substrate. Other simulations of ss-DNA–CNT hybrids revealed that the vast majority of the sampled oligonucleotides had all of their bases adsorbed. The experimental results of Tang et al. also support a hypothesis that the oligonucleotide adsorbs to a graphene surface with more than one base.

Negatively charged graphene. Since only one half of the negatively charged phosphates are presented and the functional groups of bases with positive partial charges are more accessible in the ss-DNA in comparison with the ds-DNA, a weaker repulsion between DNA and the surface can be expected. It has already been confirmed in the simulation with the smallest applied negative charge (−0.01e) on the carbon atoms of the graphene. The repulsive character of the interaction between DNA and the graphene was not observed like in the case of the simulation with the ds-DNA, but rather an attractive dispersion interaction between the closest base and the layer (a major part of the simulation) and attraction of the polar amino group of the closest cytosine and the graphene (a minor part) were seen.

When the charge of the carbons of the graphene is changed to −0.02e, the electrostatic interactions become the leading factor for the stabilisation of the DNA–graphene complex. The attraction of the amino group of the closest cytosine and the surface is crucial. Such an interaction pattern was also observed for the ds-DNA and the same charge of the graphene, where an unexpected breaking of the closest base pair to the surface was detected.

For the ss-DNA interacting with the graphene slab of charges −0.05e, two types of electrostatic attractions can be deduced: (i) the above-mentioned interaction of the closest cytosine with the surface (supplemented occasionally, especially in the second half of the simulation, by the interaction of the other bases with graphene—typically an amino group of guanine at the opposite end of DNA); and (ii) the interaction...
of the negatively charged sugar-phosphate backbone in the middle part of the ss-DNA with the counterions localised close to the surface, which became important after approx. 17 ns of the simulation.

The system containing graphene with the unit charge of \(-0.1e\) shows that the most important interaction between graphene and the DNA is mediated by the strong attraction between the phosphates and the oppositely charged, almost immobile layer of sodium cations, which are situated upon the graphene, similarly as in the case of ds-DNA. Since the ss-DNA is more flexible than ds-DNA, more phosphates are involved in the direct interaction with sodium cations than in the case of ds-DNA (the average distance of the central phosphorus atom from the surface is by 0.8 Å smaller). The bases point away from the surface, whereas ds-DNA retains its helical structure.

Positively charged graphene. Whereas for the ds-DNA the interaction motif with the graphene layer with charges of \(+0.01e\) remains similar to the one observed in the system with uncharged graphene, the ss-DNA benefits from the mutual cooperative effect of the electrostatic and dispersion interactions. This leads, approximately after 10 ns of the simulation, to a configuration where the whole ss-DNA is localised close to the surface and interacts \(\textit{via}\) phosphates (by electrostatic forces) and as well as with majority of its bases (by dispersion forces) with the surface.

When the charge of the carbons in graphene is changed to \(+0.02e\), the electrostatic attraction between the phosphates and the surface causes a parallel orientation of the DNA already in the early stage of the simulation. In addition, an electrostatic interaction of the guanine with the surface mediated \(\textit{via}\) the O6 oxygen atom of the base is observed. Occasionally, one or a few bases still interact with the surface by dispersion forces.

For the system containing graphene with a charge of \(+0.05e\) on the carbon atoms, the electrostatic forces attracting the phosphates to the surface are crucial. However, like for the ds-DNA with the same charged graphene slab, it takes about 10 ns before the parallel orientation of the DNA with the surface is reached (probably owing to the presence of a non-negligible amount of chloride anions next to the surface). Generally the relaxation time of 10–25 ns is typical for reaching the equilibrated state for most of our simulations.

As for the ds-DNA at the surface with a charge of \(+0.1e\), the ss-DNA remains extended and perpendicularly orientated.
to the graphene surface covered by an almost immobile layer of counterions (Cl\(^-\)) at the beginning of the simulation. This rather strong repulsion between the chloride anions and phosphates leads to the extension of the linker as well as the DNA, resulting in an increase of the distance of the central phosphorus atom from the surface from an initial value of 27 Å to 38 Å within a few ns of the simulation. Later, the DNA gets closer to the surface and some of the phosphate groups are able to penetrate the chloride layer starting to interact directly with the positively charged layer. This conformational motif is similar to the motif observed by Wong et al\(^{53}\). Although their system had a higher charge density (0.711 C m\(^-2\)) and more ions compared to our system, they similarly noticed oligonucleotide with its last few bases forming a curved segment and closely interacting with the surface through its phosphate groups.

A graphical representation of the above-mentioned competition between base stacking and the electrostatic attraction of the phosphate group is depicted in Fig. 8. The increasing number of adsorbed phosphate groups with the increasing positive surface charge up to +0.05e can be seen from Fig. 8 both for ss-DNA and to a smaller extent also for ds-DNA, although the competition between base stacking and the phosphate groups’ adsorption onto the surface is not as strong in the latter case.

C. Effect of the DNA sequence

Since in our study we used only one DNA sequence, the question arises: can the presented observations be applied generally on DNA, or are they specific for a given sequence? The effect of the DNA sequence can be expected to be more pronounced for neutral or low-charged surfaces. A higher ratio of purine bases can lead in the case of very flexible ss-DNA to a slightly stronger interaction with the graphene surface than for ss-DNA containing the pyrimidine-rich sequences. Sequence-specific interactions of the terminal bases are of limited practical importance, since the majority of the DNA probes used in experiments have identical bases at their ends (namely G/C, introduced to improve stability). The sequence specificity of the DNA–surface interactions likely diminishes for highly charged surfaces, where electrostatic interactions between the phosphates and the surface/ions play a dominant role.

D. Interaction of ions with the surface

So far, we have discussed the role of the ions surrounding the DNA and also interacting with the surface only qualitatively based on simulation snapshots (Fig. 4 and 7). Here, we quantify the adsorption of ions and the role of the presence of DNA in the distribution of ions.

The axial density profiles of ions interacting with the surface are given in Fig. 9. As expected, the distribution is rather independent of the presence of ds-DNA or ss-DNA because of the dominating effect of the direct surface-ion interactions. This is also confirmed by selected simulations (for charges −0.05e, 0e and +0.05e) of the graphene surface interacting with aqueous solutions of ions without DNA. Only for a neutral surface or a small magnitude (±0.01e) of the surface charges are the curves qualitatively different owing to the

Fig. 7 The typical conformations of ss-DNA after the equilibrium is reached.
Fig. 8 Changes in the averaged number of stacked bases and adsorbed phosphate groups with increasing positive surface charge. Bases were considered as stacked when N1 and N3 atoms in their heteroatomic rings were within 4.5 Å from the surface. Similarly, a distance criterion for phosphate groups was set to 5.5 Å for the phosphorus atom. Equilibrated parts of simulations were considered in analysis. Since the negatively charged surface repels DNA, the dependence is depicted only for the positively charged and uncharged slab.

Fig. 9 Density profiles of Na⁺ (solid lines, positive values) and Cl⁻ (dashed lines, negative values) as a function of distance from the graphene surface in the presence of ss-DNA, ds-DNA, and without DNA. Note the different vertical scales of the graphs.
different number of dissolved ions, including those compensating for the charge of the DNA, cf. Table 1.

For the most negative surface (charges $-0.1e$), we observed a single adsorption peak of Na$^+$ around 2 Å, which corresponds to inner-sphere adsorption of the ion. In this case, the ion is partially desolvated and directly interacts with the negatively charged carbon atoms. Charges of $-0.05e$ cause the coexistence of both inner-sphere and outer-sphere adsorbed cations, with the latter fully solvated and around 4.3 Å. The position of the inner-sphere adsorbed ions shifts to slightly larger distances—2.4 Å from the surface. For smaller magnitudes of the carbon charges, the solvation dominates over electrostatics and no inner-sphere adsorption is observed.

A very similar situation is observed for anions at positively charged surfaces. At the largest carbon charges, +0.1e, all of the Cl$^-$ adsorb as an inner-sphere complex at a height of around 2.8 Å from the graphene surface. The position of this peak is shifted to the longer distances compared to that of Na$^+$ at a negative surface because of the larger size of the Cl$^-$ ions. For a surface with +0.05e charges, the inner-sphere adsorption shifts to 3.3 Å, but it still dominates and occurs even for a surface with charges of +0.002e, although followed by a range of adsorption distances with the increasing solvation of the Cl$^-$.

The formation of strong and well-defined adsorption peaks of either ions in the extremely charged systems explains why the observed behaviour of DNA in these cases does not follow the trend seen for smaller charges. As stated above, in these cases the DNA feels the layer of adsorbed counterions and the DNA conformation and its orientation to the layer depend on its ability to penetrate the layer of counterions and establish direct contact with the surface.

We should mention that for an even slightly higher surface charge density than studied here, Wong et al. observed two adsorption peaks for Cl$^-$ separated by ~2.2 Å, but that has been attributed to sandwiching of the surface ammonium coating of their β-cristobalite surface. In our case of a nearly perfectly flat surface, the formation of single or multiple adsorption layers of ions must be exclusively assigned to interplay between the surface and solvation interactions.

Finally, the role of the ions and the surface charge in the hybridisation processes should be discussed. The environment at the solid–liquid interface is very different from the bulk solution. As a result, the thermodynamics and kinetics of the solid-phase hybridisation differ substantially from those in the bulk. One of the key factors is the presence of cations. Springer et al. studied the effect of the monovalent and divalent cations on solid-phase DNA hybridisation. The authors found that the duplex stability is substantially lower than the one reported for the bulk (but still in the range of tens of kJ mol$^{-1}$ for an oligomer). In the absence of magnesium, higher sodium concentrations stabilize the duplex. The hybridisation free energy depends linearly on the logarithm of the sodium concentration, as observed also in the bulk. However, the slope is much lower in the solid phase than in the bulk. Thus, the sodium concentration influences the duplex stability much less in solid phase hybridisation than in the bulk. Our effective sodium concentration, based on the number of ions (Table 1) and the volume of the simulation box, varies between 60 mM and 1 M.

This corresponds to the difference in the hybridisation free energy of just only 1 kJ mol$^{-1}$.

The surface hybridisation kinetics is also very different from that in the bulk. Gao et al. found that the hybridisation rate constants at the surface drop by a factor of 20–40 compared to the bulk. The authors also observed that the hybridisation rate increases with increasing salt concentration (a several fold increase with the NaCl concentration changing from 100 mM to 1 M). Peterson et al. studied the rate of DNA adsorption onto the surface and found roughly a 5-fold increase in the adsorption rate upon changing the NaCl concentration from 50 mM to 1 M. Note, however, that the time scales of DNA hybridisation are orders of magnitude longer than our simulation times. Indeed, based on the rate constants at 0.5 M NaCl reported by Gao et al., the characteristic time of the hybridisation reaction in our systems would be about 1 ms.

IV. Conclusions

As far as the local conformation is concerned, our results suggest that the ds-DNA retains its B-DNA features regardless of the presence of the graphite layer and its charge, except for terminal base pairs and steps. In contrast, the ss-DNA exhibits an ordered stacked helical structure if left free in solution, but it shows localised structural collapse when a graphene layer is present, even if it is not charged.

Under physiological conditions, a DNA molecule is negatively charged and polar, owing to the negatively charged phosphate groups in its backbone. Thus a strong influence on its orientation to the surface was expected when the charge of the layer was varied. Our simulation of both ss-DNA and ds-DNA anchored on the differently charged graphene layer fully confirmed that the orientation of the DNA is extremely sensitive to the charge density of the layer.

A. Uncharged layer

Interactions between the ds-DNA and graphene are mediated, besides by the tether, by the dispersion forces between the closest base pair. The DNA molecule is orientated more or less perpendicularly to the surface. On the other hand, interactions between the surface and the ss-DNA confirm both the experimental and theoretical results, namely that more bases than merely the first one are adsorbed to the surface because of the higher flexibility of the ss-DNA.

B. Positively charged layer

When a low charge density is applied on the graphene layer, the dispersion forces in conjunction with the weak electrostatic attraction are responsible for the interaction between the DNA and the layer. At medium positive surface charge densities, 0.12–0.3 C m$^{-2}$ (corresponding to partial charges +0.02 and +0.05e at each carbon atom of the surface), the electrostatic attraction of the phosphate group and the layer becomes undoubtedly dominant both for the ss- and ds-DNA.

For a highly positive charged surface, the situation is complicated by the presence of a high amount of chloride counterions, creating along with the graphene a bilayer, which is negatively charged at the side facing the DNA. Thus, the DNA is repelled from the surface and remains in the perpendicular
orientation to the surface at the first stage of the simulation. However, the DNA is later capable of disrupting the layer of counterions and begins to interact directly with the graphene by the phosphate groups.

C. Negatively charged layer

For the layers with low and medium negative charge densities, −0.06 to −0.3 C m⁻² (corresponding to partial charges −0.01, −0.02 and −0.05e at each carbon atom of the surface), a rather repulsive interaction was observed for the ds-DNA, whereas the ss-DNA prefers to interact with the charged graphene by its polar groups of the bases with a positive partial charge. For the systems with the highest applied negative charge density on the graphene, an oppositely charged layer of counterions was again formed. A strong attraction of the phosphates with the sodium cations was observed immediately after the beginning of the simulations both for the ss- and ds-DNA.

D. Ions

For surface charge densities in the range −0.12 to 0.12 C m⁻², the ions adsorb predominantly as outer-sphere complexes with a limited effect on the DNA-surface interactions. However, for the largest magnitudes of surface charges (−0.1, +0.05 and +0.1 C m⁻², i.e. −0.6, +0.3, +0.6 C m⁻²), the formation of a counterion inner-sphere layer becomes a key factor in reversing the trends and effective charge of the surface. Being a smaller ion compared to Cl⁻, Na⁺ solvation is stronger, which leads to a mixed adsorption of Na⁺ at a surface charge density of −0.3 C m⁻² in two distinct inner-sphere and outer-sphere geometries.

E. Hybridisation

The molecular dynamics simulations performed do not show due to different time scales the process of DNA hybridisation directly. However, important indirect indices have been observed showing how the proximity of the graphene surface may influence the formation or melting of the DNA duplex. The practical applications of hybridisation with surface-bound probes depend not only on the stability of the duplex formed but also on other parameters such as hybridisation kinetics, sensitivity and selectivity, which, in this case, become also functions of the parameters characterising the surface and its interactions with the DNA.

We have demonstrated that hybridisation with surface-bound probes could benefit from both electrostatic and stacking interactions between the DNA and the graphene layer. As expected, the B-form structure of the double helix itself is not affected by the vicinity of the moderately charged surface, in agreement with the experimental results. One exception to this rule was observed, namely the dangling end created on the ss-DNA prefers to interact with the charged graphene by its polar groups of the bases with a positive partial charge. However, the DNA is later capable of disrupting the layer of counterions and begins to interact directly with the graphene by the phosphate groups.

The application of an attractive electric field could in principle solve a serious problem of microarray technology—the slow hybridisation rates. On the other hand, the multiple stacking interactions between the ss-DNA bases and graphene that were observed on a positive surface up to a charge density of −0.1 C m⁻² represent a serious problem for hybridisation. Depending on the surface charge, the accessibility of the probes is reduced and the double-helix formation is slowed down if not stopped. This result clearly shows why the increase in the surface layer concentration of the target, created by the attractive electric field, which should accelerate the hybridisation kinetics, is not directly reflected by the overall hybridisation rate owing to the steric hindrance when the probes are collapsed on the surface of the chip. It is well known that hybridisation depends on many parameters such as temperature, probe and target lengths, on the composition of the buffer, on zwitterions and on the surface chemistry in the presence of electric fields. Our results elucidating the influence of the surface were obtained with a short oligonucleotide model. Similar surface-effects (steric hindrance, electrostatic and stacking interactions with graphene) can be expected with mutually non-interacting long oligonucleotides (> 30 nucleotides). The behaviour of mutually interacting probes in the so-called brush region of surface concentrations may differ from our results and should be treated separately.

The model system considered in our work is interesting for practical applications not only for its potential to accelerate hybridisation. The graphene properties are similar to graphite, a widely used material. Graphite, being composed of covalently bonded sheets of graphene, in principle leads to a bit more longer-ranged vdW interactions (coarse-graining of interaction with a single plane leads to 10-4 Steele potential, while that with half-space bulk material to 9-3 potential), however considering the strong and long-range effect of electrostatics, the effect of the difference between graphene and graphite on observed trends is not expected—as long as the surface charge density is the same.

Graphene is able to quench the fluorescence of DNA attached fluorescent labels effectively. The above-mentioned behaviour can be used for the preparation of new graphene-based microarrays with dye attached to the probe instead to the target DNA, where the hybridisation is detected by fluorescence intensity changes caused by the changing distance of the fluorescent label from the quenching surface.

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