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Optical Extinction Combined with Phase Measurements for Probing DNA−Small-Molecule Interactions Using an Evanescent Waveguide Biosensor

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We demonstrate the use of both optical extinction and phase measurements for probing the interactions between DNA and small molecules by dual polarization interferometry. On binding to DNA at the interface, mitoxantrone (MTX) and methylene blue (MB) induced reversible concentration-dependent optical extinction due to light absorption, which clearly revealed the association and dissociation of small molecules with DNA in real time. The binding constants of MTX−DNA and MB−DNA determined from the masses derived from optical extinction are 1.8×10^5 and 4.2×10^4 M^{-1}, respectively, and shown to be buffer salt concentration-dependent. Apart from optical extinction, phase measurements reflected the overall change of the interaction; namely, a combined result of the binding of small molecules and any changes in DNA structure. The masses derived from phase could be very different from those derived from optical extinction. The structural changes detected by phase measurements showed a contraction and densification of DNA upon intercalation by MTX or MB. The combination of optical extinction and phase measurements allows a detailed understanding of the interaction process.

Investigations of DNA−small-molecule interactions are important to understand the chemical basis for the antitumor mechanisms of drugs and to use this understanding to design more efficient drugs targeted at DNA.[1] Upon binding some small molecules to DNA, the DNA may immediately undergo significant changes in its structure and mechanical properties which can interfere with replication and gene expression in vivo and a number of other biological processes.[2] Therefore, it is of particular interest to study both the structure and kinetics of DNA−small-molecule interactions. A variety of methods have been applied for this purpose. For instance, X-ray diffraction, nuclear magnetic resonance, and circular dichroism spectroscopy provide particular structure information.[2−4] Surface plasmon resonance (SPR)[5−7] and quartz crystal microbalance[8] measurements can give kinetic responses. However, it is still a challenge to get both structural information and kinetics simultaneously. Recently, we have applied dual polarization interferometry (DPI) to study DNA−small-molecule interactions.[9] Owing to the DPI's ability to simultaneously determine the thickness, density, and mass of adlayers in real time,[10−16] both the structural changes in DNA and the kinetics of the interactions can be obtained.

DPI is an evanescent waveguide technique in which light travels down adjacent sensing and reference waveguides and diffracts at the output to give a Young's interference pattern in the far field, as shown in Figure 1.[10,11] The parameters that characterize the interference pattern include the position of the interference fringes (phase) and the sharpness of the interference fringes (contrast) within a diffraction envelope. Although both DPI and SPR use the evanescent field to detect changes at or near

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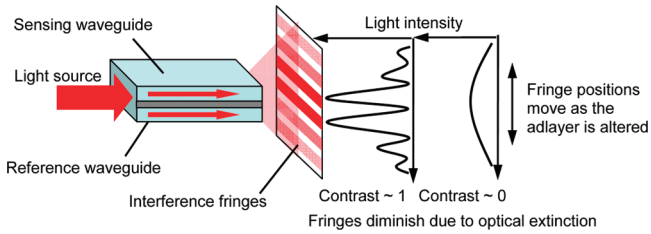

Figure 1. Schematic of the classical Young oscillating fringe pattern from the sensing and reference waveguides. Fringe positions can be affected by changes in the RI (phase shifts); the amplitudes of fringes can be reduced by optical extinction (contrast losses).

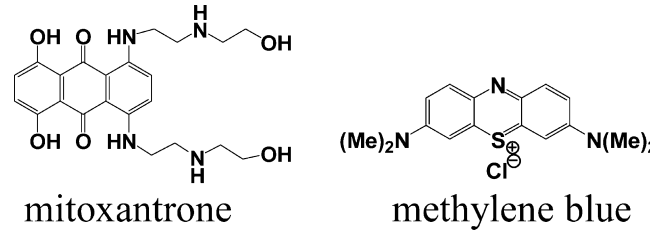

Figure 2. Molecular structures of mitoxantrone (MTX) and methylene blue (MB).

the surface, SPR is restricted to the transverse magnetic (TM) polarization, but DPI supports both a TM and a transverse electric (TE) polarization mode, which generate different evanescent fields at the sensing surface. The evanescent field is highly sensitive to events in the vicinity of the sensing surface,[13,14,17] where the evanescent field is affected by the real part as well as the imaginary part of the RI.[18] (The imaginary part of the RI relates to the absorbance of the adlayer.)

DPI can measure changes in both the real and imaginary parts of the RI by detecting changes in the position and amplitude of the interference pattern (i.e., phase and contrast, respectively). The phase measurements provide the thickness and density values of the adlayer, which are primarily used in DPI analysis. The contrast provides a relative measure of the amount of light passing down the sensing waveguide as compared with the reference waveguide. In an idealized DPI when no light is lost from the sensing surface, the contrast is 1. When all the light is lost from the sensing waveguide, the contrast is 0, and the fringes disappear (as shown in Figure 1). A loss in contrast can be described as an increase in optical extinction. This optical extinction could be due to light absorption, light scattering, or radiation losses, which are generally negligible in waveguides without curve.[17−19]

Boudjemline et al. have used contrast to monitor the early stages of protein crystallization.[20] It was found that as the protein formed a crystalline structure at the interface, light from the sensing waveguide was lost through scattering or other optical effects.[20] However, the optical extinction due to light absorption has not been reported in existing DPI applications. Compared with phase alone, the combination of optical extinction and phase measurements may allow the introduction of optical absorption analysis into the DPI method, which would provide detailed information about absorbing molecules at the interface as well as real-time changes of the whole adlayer structure and mass. In other words, the light absorption of molecules could be measured and utilized in DPI analysis, which together with DPI's ability to determine the thickness and mass from the phase measurements provides very significantly more information than mass-based biosensors alone. Looking ahead, there is the potential to further extend the technique so that when the molecules taking part in interactions at the interface have different absorption properties, it may be possible to selectively tailor the wavelength of light to the absorption profile of the molecule of interest. This would enable the optical extinction measurement to detect the molecule of interest selectively and quantitatively with little interference from molecules in bulk solution and other nonoptically absorbing molecules in a manner unparalleled by other biosensor techniques.

Here, we show the combination of optical extinction and phase measurements for probing DNA−small-molecule interactions. Mitoxantrone (MTX) is one of the anthracyclines that have been widely used for the treatment of cancers,[21,22] whereas methylene blue (MB) belongs to the phenothiazinium dye, which has potential applications in anticancer therapy.[23] Both MTX and MB shown in Figure 2 can bind DNA through intercalation, which is one of the most common noncovalent interactions between DNA and small molecules having aromatic planar components, such as anthracyclines, phenothiazines, acridines, etc.[1,4,21,23,24] These DNA intercalators have been used extensively as antitumor, antineoplastic, and antibiotic agents, and new derivations continue to be developed as potential drugs.[1,22] In our experiments, the dominant cause of optical extinction was the absorption of light by the MTX and MB bound to the DNA at the interface, where the contribution from bulk solution and scattering was negligible. The mass change during the interaction was calculated from the extinction coefficient of the layer as well as from the phase change. The mass of the absorbing molecule could be thus separated from the total phase derived mass changes of the DNA. DNA structural changes observed with MTX and MB were consistent with intercalation that was found to induce contraction and densification of the DNA layer in our previous DPI study.[9] Therefore, the combination of the optical extinction and phase measurements can reveal more information of DNA−small-molecule interactions than the phase alone.

EXPERIMENTAL SECTION

Materials. Mitoxantrone, DNA sodium salt from calf thymus type I, and polyethylenimine (PEI) (M_w = 750 000) 50% (w/v) aqueous solution were purchased from Sigma Chemical Co. Methylene blue and 3-(aminopropyl)triethoxysilane were purchased from Acros Organics. Other agents used were analytical grade. The purity of DNA was checked by determining the ratio of absorbance at 260 nm to that at 280 nm. The A260/A280 ratio was about 1.8, indicating that the DNA was sufficiently free of protein. All chemicals were used without

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any further purification. Milli-Q grade (Millipore, Billerica, MA) water was used for the preparation of all solutions. The phosphate-buffered saline (PBS, pH 7.4) solutions were prepared with 10 mM NaH_2PO_4−Na_2HPO_4 and 150 mM NaCl. Buffers were filtered and degassed before use.

DPI Experiments. DNA immobilizations and DNA−small-molecule interactions were investigated in real time using DPI (*Ana*Light Bio200, Farfield Group Ltd., Crewe, U.K.). The details of the instrument and theory have been described previously elsewhere.[10,11] Unmodified or amine modified *Ana*Chips (FB 80) were used. All solutions used were prepared using 10 mM PBS (pH 7.4, 150 mM NaCl), and DPI experiments were performed at 20 °C in 10 mM PBS (pH 7.4, 150 mM NaCl), unless otherwise stated. The sensor chips and the running buffer were calibrated using 80% (v/v) ethanol/water solution and water before each DPI measurement.

After calibration, the flow rate was changed to 100 μL min^{-1}. On an unmodified chip, a PEI (0.2 mg/mL) solution in PBS was injected over both channels for 1 min and 45 s and then incubated for 5 min before returning to PBS. After a steady baseline was reached, a DNA (0.1 mg/mL) solution in PBS was injected over the experiment channel for 1 min 45 s and incubated for 5 min before returning to PBS. On an amino-modified chip, the same DNA was injected over the experiment channel as above. After the DNA/PEI or DNA/amino surface had stabilized, the flow rate was changed to 50 μL min^{-1}. To interrogate DNA−small-molecule interactions, MTX or MB solutions in the range of 2.0−20 μM were injected over both channels for 4 min.

RESULTS AND DISSCUSSION

Optical Extinction Induced by Small Molecules at the Interface. The experimental data for interactions of MB and MTX with DNA were recorded in both TM and TE polarizations. The evanescent field of two polarizations has different intensities and decay rates within a layer; therefore, different responses in each polarization are usually seen. Such differences between TM and TE modes is predominantly affected by the RI and the presence of birefringence caused by an anisotropic layer, which can provide a measure of degree of molecular alignment.[25,26] Because most of the layer structures were isotropic in our experiments, the figures below show only TE mode data for clarity, unless otherwise stated.

Figure 3 shows the reversible optical extinction induced upon the injection of MB solution over the DNA surface, consistent with the noncovalent intercalation of MB to DNA. Before the injection of MB onto the DNA surface, there was a classical Young's fringe pattern (see inset A in Figure 3); however, upon the injection of MB, the fringe pattern was abruptly lost (see inset B in Figure 3). Similar optical extinction was also observed during the injection of MTX over DNA surface. However, another DNA-binding small molecule, ethidium bromide (EtBr) did not induce any optical extinction, even at a relative high concentration.[9] The absorption spectra of MB, MTX, and EtBr are shown in Figure 4. As can be seen, both MB and MTX have a large absorbance at

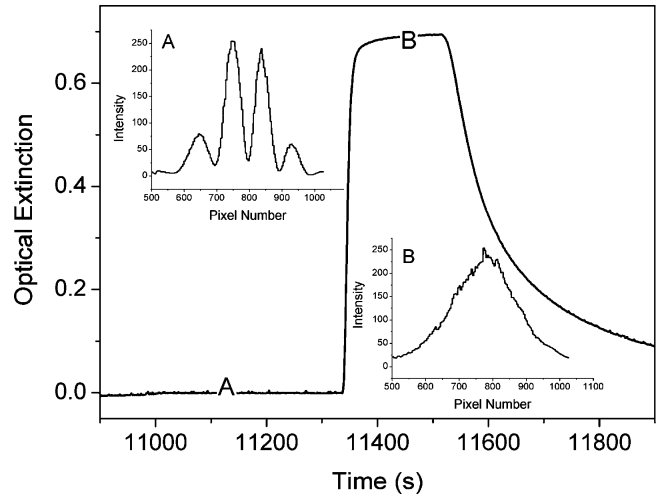

Figure 3. The real-time optical extinction upon the injection of 25 μM MB solution over the DNA/amino surface and typical DPI fringe patterns (insets) at points A and B.

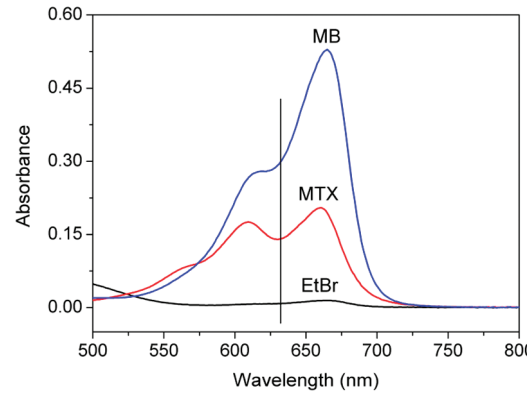

Figure 4. The absorption spectra of 10 μM MB, MTX, and EtBr in PBS buffer. The wavelength of the DPI light source at 632.8 nm is indicated by the vertical line.

632.8 nm, whereas the absorbance of EtBr is insignificant. Furthermore, because the EtBr also binds via intercalation, with similar structural changes but no loss of contrast, scattering can also be excluded as a contributing factor to the extinction for these molecules.

Within these experiments, extinction caused by molecules bound at a surface dominates because the solution concentration of MB (or MTX) above any layer is low. This is evident when MB or MTX is flowed over surfaces that attract and bind these small molecules or repel them. As seen in Figure 5, MB or MTX at 10 μM induced obvious optical extinction with the attractive DNA/PEI layer (a), DNA/amino layer (b, c), and the bare chip surface (d). In contrast, the repulsive PEI (e) and amino (f, g) surfaces showed little optical extinction.

The surface of the bare chip is predominantly silica and has a weak negative charge; consequently, a small amount of MB could be adsorbed due to electrostatic and hydrophobic interaction. The weak attraction resulted in a small optical extinction (line d in Figure 5). The PEI and the amino surfaces were positively charged and electrostatically repelled the positively charged MB or MTX, which is protonated at the pH used here.[27] Therefore, there was very little binding of MB or MTX at the interface, and negligible optical extinction was observed (line e−g in Figure 5).

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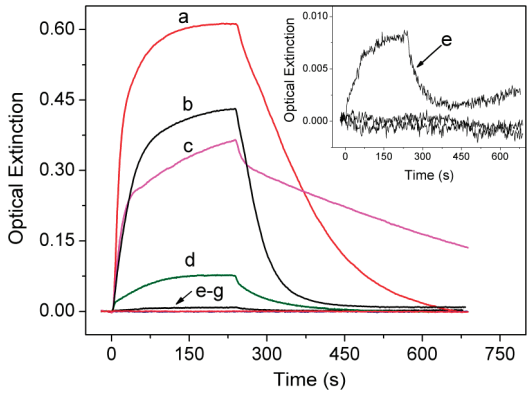

Figure 5. The optical extinction during the injection of 10 μM MB over the DNA/PEI layer (a), the DNA/amino layer (b), bare chip surface (d), the PEI surface (e), and the amino surface (f), or 10 μM MTX over the DNA/amino layer (c) and the amino surface (g). The RI and mass of the DNA layer on PEI: 1.382, 2.05 ng/mm^2; the RI and mass of the DNA layer on amino surface: 1.379, 1.36 ng/mm^2.

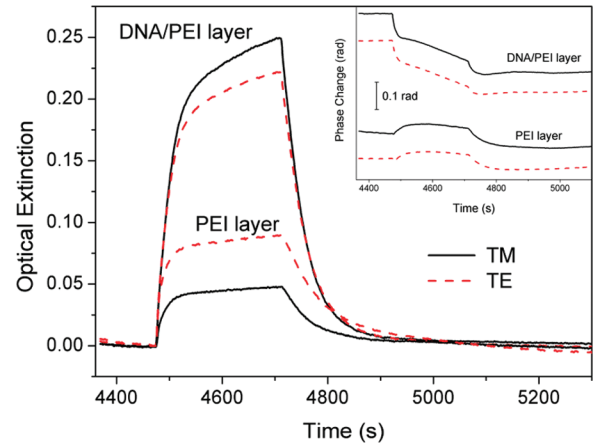

Figure 6. The optical extinction and phase changes (inset) induced upon the injection of 20 μM MB over DNA/PEI and PEI surfaces, recorded in TM and TE polarizations, respectively.

The DNA layers deposited on the PEI or amino surface had a very similar density (with a RI of ~1.38). However, the mass of DNA deposited on the PEI (~2.18 ng/mm^2) was larger than that on the amino surface (~1.36 ng/mm^2). This may be due to differences in the charge density and structure of the PEI and amino surfaces. The optical extinction due to MB on the DNA/PEI (line a in Figure 5) was also larger than that on the DNA/amino surface (line b in Figure 5), which indicates a larger amount of MB bound to the DNA/PEI surface owing to the greater amount of immobilized DNA. Similar to MB, 10 μM MTX also induced obvious optical extinction on the DNA/amino surface (line c in Figure 5).

Although the PEI surface resisted significant adsorption of MB, a slight optical extinction can still be seen in the enlarged view (the inset in Figure 5). This might be due to a small amount of MB that was able to penetrate the PEI layer and bind to the chip surface. The adsorbed PEI layer is expected to be more open and flexible than the amino-silaned layer, which would prevent penetration of MB to the underlying chip surface. If this is true, reducing the density of the PEI layer would allow more penetration, and hence, a larger optical extinction should be observed from the MB. This is confirmed and shown in Figure 6, where the optical extinction in both polarizations is plotted. The RI of the PEI layer in Figure 6 is about 1.41, less dense than the PEI layer having a RI of 1.46 in Figure 5. Nonspecific adsorption of MB to the PEI layer was unlikely to happen because of the electrostatic repulsion between MB and PEI, and it can be further excluded by the comparison of optical extinction between TM and TE. The polymer layer structures in these experiments are isotropic, with the polymeric PEI and DNA molecules randomly oriented on the surface. If the MB when it binds is also similarly randomly oriented, then the optical extinction observed in TM and TE polarizations would give similar responses, with the magnitude of the response in TM polarization slightly larger than the TE polarization. This is, indeed, observed and is shown in Figure 6. However, a much larger TE than TM response was observed when MB was injected over the PEI layer. It indicates that the MB was not randomly oriented with respect to the waveguide surface (but probably with its aromatic structure parallel to it). This is consistent with reported observations of MB adsorbed onto a silica surface with its long axis titling toward the surface plane at an angle of <30 degrees.[28] Although optical extinction observed for the DNA/PEI layer was obvious and reversible, the phase changes were unclear and continuously decreased while MB flowed over the layer. This might be due to the unstable and open structure of the DNA/PEI layers, which also significantly affected the effective RI, thereby changing the phase in addition to the binding of MB itself.

Combination of Optical Extinction and Phase Measurements for Probing DNA−Small-Molecule Interactions. In general, DPI experiments are concerned with phase measurements because little optical extinction would be induced by most biomacromolecules and polymers. However, since the optical extinction was induced by MTX and MB, it is of interest to compare both the phase change and the optical extinction induced by the small molecules upon binding to DNA at the interface.

Comparison of the Real-Time Changes between Optical Extinction and Phase. Figure 7 shows the real-time optical extinction induced by MTX or MB on the DNA/amino and the amino surfaces. MTX and MB induced little optical extinction on the amino surface, even at relatively high concentration due to electrostatic repulsion from the amino surface, as discussed above. On the DNA/amino surface, the optical extinction increased with increasing concentration of MTX or MB, which is due mainly to the absorption of small molecules bound at the interface and therefore should reflect the binding of the small molecules to the DNA. Interestingly, the distinct profiles of MTX−DNA and MB−DNA indicate different kinetics, which might be closely related to their interaction modes. Although both MTX and MB bind to DNA through intercalation, MB typically intercalates between DNA base pairs,[23] whereas MTX, which has a planar anthraquinone ring and two nitrogen-

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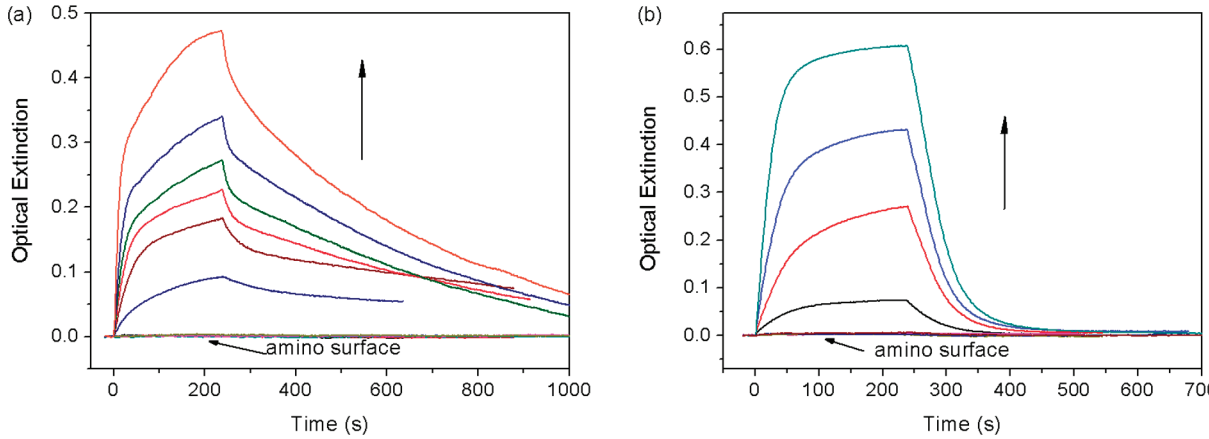

Figure 7. The optical extinction during the injections of (a) 2, 4, 6, 8, 10, and 20 μM MTX and (b) 2, 6, 10, and 20 μM MB over the DNA/amino and amino surface. The arrows indicate the increase in the concentration.

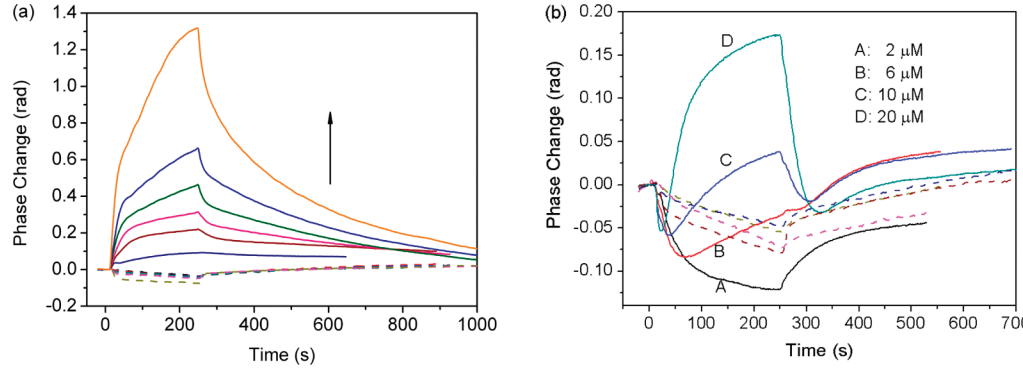

Figure 8. The phase changes during the injections of (a) 2, 4, 6, 8, 10, and 20 μM MTX and (b) 2, 6, 10, and 20 μM MB over the DNA/amino (solid lines) and amino surface (dashed lines). The arrows indicate the increase of concentration.

containing side chains (as shown in Figure 2), was reported to partially intercalate DNA with its two side chains lying in the grooves of DNA.[21] This multiple binding mode might result in more stable binding and harder dissociation of MTX from DNA. Under similar conditions, MTX did not seem to reach equilibrium in the way that MB did. This might also be due to more stable binding, slower dissociation, and more complex binding of MTX with DNA.

Figure 8 shows the phase changes induced by MTX or MB on the DNA/amino and the amino surface. Both MTX and MB induced small and similar phase changes on the amino surface. The phase changes induced by MTX on the DNA/amino surface seemed similar to the real-time optical extinction and followed the same trend (as shown in Figures 7a and 8a), indicating the binding of MTX to DNA dominated the whole interaction process. However, for MB, the phase and optical extinction had a different relationship. The phase decreased at low concentrations of MB or at the beginning of high concentrations (as shown in Figure 8b). At first, we speculated the phase decrease was due to desorption of DNA from the surface. However such phase changes were always observed with MB on the DNA/amino surface and they seemed to be somewhat reversible, making it unlikely to be solely due to irreversible desorption of DNA.

To explain it, we might have to look at the origin of a phase change. A phase change, unlike optical extinction only resulting from small molecules, could be contributed by any changes in the effective RI experienced by the evanescent wave component of the light,[29] including the binding of small molecules to DNA and other changes, such as the uptake or release of counterions and water molecules in the adlayer. Counterions and water molecules are important for the maintenance of DNA structures. For instance, the Na^+ ions commonly used in experiments can penetrate the DNA interior and condense around the DNA exterior, whereas the counterion's distribution is directly related to DNA conformational preferences.[30] The binding of small molecules to DNA might more or less affect the counterion's distribution or even displace part of the counterions. It is commonly known that intercalation can induce large conformational changes in DNA.[1] Therefore, the unusual phase changes during the injection of MB might be due to significant structural changes in the DNA layer rather than merely the binding of MB itself. However, unlike the intercalation, the groove binding usually has less impact on DNA conformation;[1] thus, there was no such dramatic phase variations due to structural changes during the partial intercalation and groove binding of MTX to DNA. This can be further confirmed by the structural changes in DNA measured by DPI (section C).

Masses Derived from Optical Extinction and Phase. The mass changes during the interactions can be calculated from

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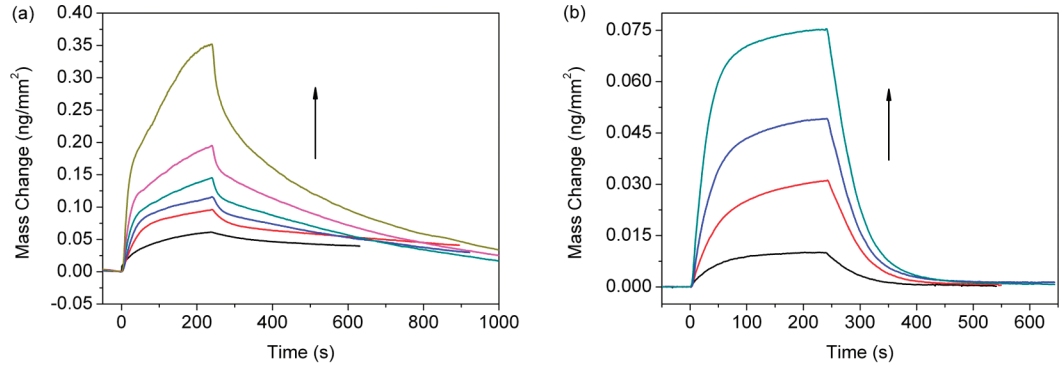

Figure 9. The mass changes derived from optical extinction during the injections of (a) 2, 4, 6, 8, 10, and 20 μM MTX and (b) 2, 6, 10, and 20 μM MB over the DNA/amino (the mass changes were the average of TE and TM polarizations). The arrows indicate the increase in the concentration.

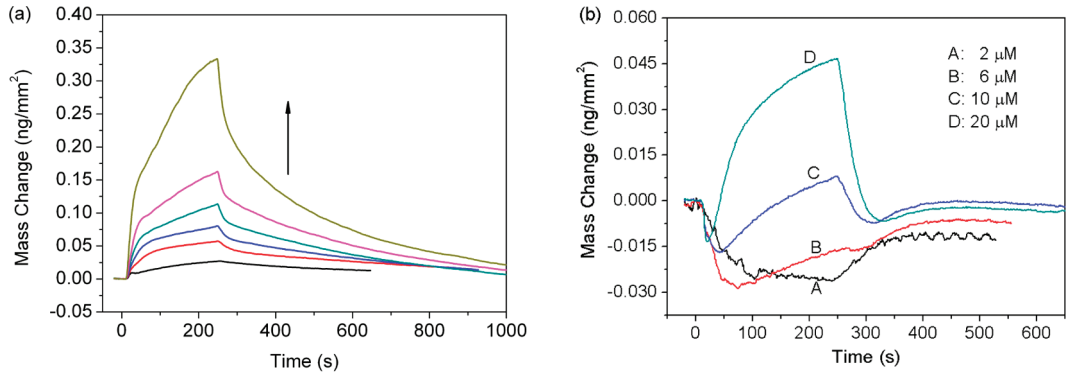

Figure 10. The mass changes derived from phase changes during the injections of (a) 2, 4, 6, 8, 10, and 20 μM MTX and (b) 2, 6, 10, and 20 μM MB over the DNA/amino surface with the control surface subtracted. The arrows indicate the increase in the concentration.

both phase and optical extinction, respectively (see the Supporting Information). It should be noted that the masses derived from phase measurements need to be scaled according to the refractive index increments (RIIs) of MTX and MB, as most small molecules have different RIIs from DNA, usually in the range of 0.2−0.3.[31] Here, a RII of 0.25 for both MTX and MB was used for the correction, since only a rough comparison between the mass derived from phase and optical extinction is made, and this assumes there is no significant contribution from anomalous dispersion.

Figure 9 shows the mass changes derived from optical extinction during the injections of MTX or MB, indicating reversible binding of MTX or MB to DNA, consistent with intercalation between the small molecules and DNA. Figure 10 shows the mass changes derived from phase during the interactions. It is interesting to find that the mass changes derived from optical extinction and phase are similar for the MTX−DNA interaction (as shown in Figures 9a and 10a). If the binding of a small molecule to DNA did dominate the whole interaction process, as discussed above, the mass derived from optical extinction should be similar to that derived from phase, as observed with MTX. However, the two masses are very different for the MB−DNA interaction (as shown in Figures 9b and 10b). The mass changes derived from the phase (Figure 10b) indicate that some molecules were released from the surface in addition to the binding of MB to DNA, and therefore, the overall mass changes were not just due to the binding of MB. Although it is not currently possible to compare the exact values of the mass changes derived from optical extinction and phase, we speculate that the mass gain due to the binding of MB to DNA might be partly counteracted by the release of molecules such as counterions from the DNA.

Structural Changes from Phase Measurements. The structural changes of DNA induced by MTX or MB were obtained from the phase measurements. Figure 11 shows representative changes in the density (RI) and thickness of the DNA layer upon exposure to MTX or MB. The increase in the RI and decrease in the thickness suggest that the DNA layer became denser and thinner upon binding MTX or MB, respectively. This is consistent with the structural change of DNA observed in our previous study of EtBr−DNA interactions.[9] MTX, MB, and EtBr all can interact with DNA through intercalation, which has been reported to be able to induce contractions of DNA.[32,33] It is worth noting that the thickness decrease induced by MB was generally about 10 times that induced by MTX at the same concentration (as shown in Figure 11). This might be due to the difference between MB−DNA and MTX−DNA interactions. As discussed above, MB intercalates into DNA, but MTX interacts with DNA through partial intercalation and groove-binding.[21,23] The groove-binding usually does not induce as large conformational changes in DNA as intercalation.[1] Therefore, the binding of MTX to DNA might only result in a smaller DNA

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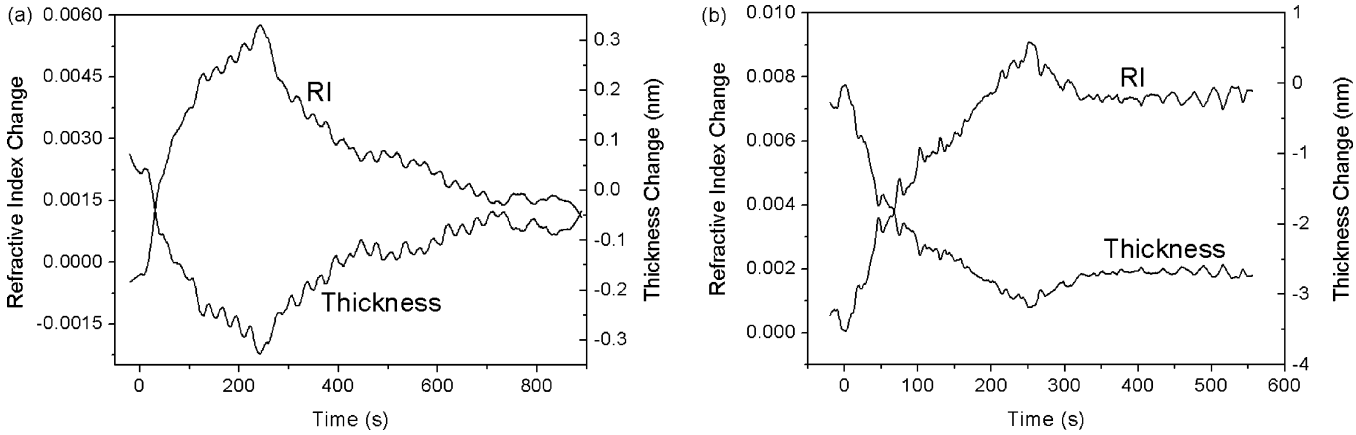

Figure 11. The RI and thickness changes derived from phase changes during the injection of (a) 6 μM MTX; (b) 6 μM MB over the DNA/amino surface.

structural change. Compared with MTX, the structural change of DNA induced by MB seemed to be larger and irreversible. We speculate that DNA could significantly rearrange on the surface when experiencing a large structural change, which could then make the structural change due to the binding of MB to DNA irreversible.

Binding Constants and Kinetics of Small Molecules to DNA. The binding, association and dissociation rate constants of the small molecules to DNA were calculated using the masses derived from optical extinction. For MTX−DNA interaction, the binding constant is $K_a = 1.8 \times 10^5$ M^{-1}, with an association rate constant of $k_a = 364$ M^{-1} s^{-1} and a dissociation rate constant of $k_d = 0.002$ s^{-1}. For MB−DNA interaction, the respective values are $K_a = 4.2 \times 10^4$ M^{-1}, $k_a = 876$ M^{-1} s^{-1}, and $k_d = 0.021$ s^{-1}. The binding of MTX to DNA is stronger than MB. This might be due to the additional groove-binding being more stable than intercalation.[1] Mao et al. measured the equilibrium and rate constants of MTX to surface-immobilized DNA using piezoelectric quartz-crystal-impedance measurements (PQCI), and obtained $K_a = 4.7 \times 10^5$ M^{-1}, $k_a = 66$ M^{-1} s^{-1}, and $k_d = 0.00014$ s^{-1}, respectively.[27]

The differences could be attributed to different experimental methodologies and experimental conditions. Although both PQCI and DPI experiments were based on tethering the DNA on the sensing surface, the PQCI experiments involved incubating the DNA modified surface in the buffer containing MTX, whereas in the DPI experiments, the MTX solution continuously flowed over the DNA surface. However, the most likely factor is the buffer composition; in particular, the salt concentration (see below). The PQCI work used buffer with a very low ionic strength, which might have resulted in the higher binding constant. For small molecules with positive charge, such as MTX and MB, electrostatic interaction with the negatively charged DNA backbones contributes significantly to the stabilization of the complexes.[23,27] High concentrations of NaCl can effectively screen the negative charges of DNA backbones, thereby reducing the electrostatic interaction and resulting in a lower binding constant.[30] Zhang et al. reported the binding constant of MB−DNA to be 1.89×10^4 M^{-1}, lower than the value obtained here.[23] In this case, a buffer containing 500 mM NaCl was used, which is much higher than the 150 mM NaCl used here. To prove this, the binding of MB was measured as a function of salt concentration. This is shown in the Supporting Information, with the affinity varying between the 1.89×10^4 M^{-1} reported value at 500 mM salt to 9.33×10^4 M^{-1} at 50 mM salt shown in Section 4 of the Supporting Information.

CONCLUSIONS

Optical extinction was investigated as an additional source of information to characterize DNA−small-molecule interactions. MTX and MB induced different degrees of optical extinction on binding to surfaces of varying affinities. On binding to DNA at the interface, both MTX and MB induced reversible and concentration-dependent optical extinction, which reflects mainly the binding of the small molecules to DNA. In contrast, phase changes reflect the overall changes during the interactions. The masses derived either from optical extinction or from phase changes were compared. For MTX, the two masses were comparable, although the mass derived from phase was just an estimated value, indicating the binding of MTX to DNA dominated the interaction. For MB, significant differences between the two masses were observed, indicating that in addition to the binding of MB to DNA, other factors, such as the release of counterions, also contributed to the overall changes. The binding, association and dissociation rate constants of MTX−DNA and MB−DNA were determined from the masses derived from optical extinction and compared with previous reports. The structural changes of DNA resolved from phase changes indicated a contraction and densification of DNA upon intercalation by MTX or MB, consistent with our previous study.[9]

To date, phase measurements by DPI have been used mainly to investigate various interactions. We have shown here the effect of optical extinction due to light absorption by small molecules bound at the interface. In the future, multiple wavelengths could be used with the standard DPI design to enable measurements over a wider range of molecular systems, including dyes, pigments, and liquid crystals, with broader application beyond biological interactions to structured molecular interfaces of relevance in areas of nanoscience, molecular electronics, etc. (Indeed, the current waveguide structure can theoretically already support a wavelength range of 570−1050

nm which could be further extended by changing the waveguide design.)

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SUPPORTING INFORMATION AVAILABLE

The experimental details for amino modification of chips, DPI data analysis, the measurements of the molar extinction coefficients for MTX−DNA and MB−DNA, and comparison of affinities at different salt concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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