Supplementary Materials for the paper entitled as "Optical Extinction Combined with Phase Measurements for Probing DNA–Small-Molecule Interactions Using an Evanescent Waveguide Biosensor"

Section 1: Amine Modification of Chips.

The unmodified *Ana*Chips (FB 80, Farfield Group Ltd., Crewe, UK) were first cleaned by immersion in piranha solution (70% H_2SO_4, 30% H_2O_2) for 10 min and sequentially rinsed by sonicating in ultrapure water and ethanol for 5 min. The cleaned chips were immersed in a 5% solution of 3-(aminopropyl)triethoxysilane in ethanol for 4 h, followed by thorough rinsing in absolute ethanol and dried by N_2, to provide the amino surface.

Section 2: DPI Data Analysis

The real components of the propagation constants are obtained from the change in phase in both TE and TM polarizations. The imaginary component of the propagation constant is obtained via the behaviour of the contrast in one polarization. The three components of the propagation constants in two polarizations are used to fit the thickness, RI and the extinction coefficient of the layer. A model that solved Maxwell's equations for a multiple stack dielectric system was used to calculate the properties of the layer formed. The model assumed that absorption was the dominant cause of light lost in the sensing waveguide and that the buried reference waveguide was unaffected by absorption.

The thickness and real component of the RI were combined to provide the mass per unit area according to de Feijter formula:[1]

$$m_L = \tau_L(n_L - n_{buffer})/(dn/dc) \quad (1)$$

where m_L is the layer mass per unit area (ng/mm^2); τ_L is the layer thickness (nm); n_L, n_{buffer} is the RI of the layer and the bulk solution, respectively; dn/dc is the RI increment (RII) (cm^3/g). A RII of 0.175

cm^3/g for PEI and DNA was used here according to Lee et al.[2] The RIIs for small molecules were estimated according to the literature[3] and used to correct the mass derived from phase.

With the use of the Beer Lambert Law and the known molar extinction coefficient of the small molecule, the measured extinction coefficient and thickness of the layer the mass of the small molecule within the layer per unit area can be calculated via Eq. 2:

$$m_d = (4\pi M_w \tau_L k_L)/(\varepsilon \lambda_0 \ln 10) \quad (2)$$

where m_d is the total small molecule mass per unit area (ng/mm^2); M_w is the molecular mass, 517.4 g/mol for MTX and 319.9 g/mol for MB; τ_L is the layer thickness (nm); k_L is the extinction coefficient of the layer; ε is the molar extinction coefficient, 12 067 $cm^{-1}M^{-1}$ for MTX–DNA and 27 033 $cm^{-1}M^{-1}$ for MB–DNA (The measurements of ε are given in Section 3); λ_0 is the free space wavelength, set at 632.8 nm for this work.

Section 3: Measurements of the Molar Extinctions

The molar extinction coefficients for MTX–DNA and MB–DNA at 632.8 nm were measured by Cary 50 UV–vis spectrophotometer (Varian, USA) equipped with a quartz cell of 1 cm path length. The phosphate-buffered saline (PBS, pH 7.4) solutions were prepared with 10 mM NaH_2PO_4–Na_2HPO_4 and 150 mM NaCl, and used for all the measurements. The MTX or MB stock solution of 2 mM was diluted to >10 μM in PBS just before use. The concentration of DNA stock solution (base pairs) was spectroscopically determined using a molar absorption coefficient of 13 200 $cm^{-1}M^{-1}$ at 260 nm after dilution in PBS. The MTX–DNA or MB–DNA solution was obtained as follows: a 15 μL of MTX or MB stock solution was added to 2900 μL PBS; a 100 μL of DNA stock solution was then added; the mixture containing 10 μM MTX or MB and 100 μM DNA was gently shaken for at least 5 min to ensure full interaction between the small molecules and DNA. 10 μM MTX or MB was selected according to the concentration range (2–20 μM) used in the DPI experiments. A relatively high

concentration of DNA (100 μM) was selected to allow the intercalation of MTX or MB into DNA as similar as to that in the DPI experiments.

The absorbance of MTX–DNA or MB–DNA solution at 632.8 nm was measured to calculate the molar extinction coefficients. The molar extinction coefficients for MTX–DNA and MB–DNA at 632.8 nm are 12 067 and 27 033 $cm^{-1}M^{-1}$, respectively.

Section 4: Comparison of Affinities at Different Salt Concentrations

To show the influence of salt concentration on the affinities of small molecules and DNA, the affinities of MB–DNA were measured at 50, 150 and 300 mM NaCl concentrations.

Table S1. The affinities of MB−DNA measured at different salt concentrations.

NaCl concentration	50 mM[a]	150 mM[a]	300 mM[a]	500 mM[b]
$K_a \times 10^{-4}$ (MB−DNA) M^{-1}	9.33±0.85	5.75±0.43	3.96±0.53	1.89

[a] different NaCl concentrations in 10 mM NaH_2PO_4−Na_2HPO_4, pH 7.4. The affinities of MB−DNA were obtained directly from optical extinction measurements (n = 8).
[b] 50 mM Tris, pH 7.1 and 500 mM NaCl in the literature 4.

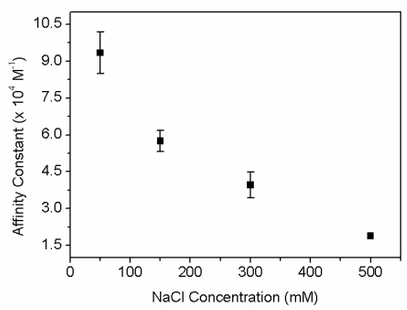

Figure S1. The affinities of MB−DNA as a function of NaCl concentration.

Reference:

1. Feijter, J. A. D.; Benjamins, J.; Veer, F. A. *Biopolymers* **1978**, *17*, 1759-1772.

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4. Zhang, L. Z.; Tang, G. Q. *J. Photochem. Photobiol. B* **2004**, *74*, 119-125.