Supplementary Information
Characterization of the binding interactions between EvaGreen dye and dsDNA

L. C. T. Shoute and G. R. Loppnow*
Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2 Canada

Hill analysis: To gain insight into the binding interaction between the duplex DNA (poly-dA_{17}•poly-dT_{17}) and EG, we have prepared and recorded the fluorescence spectra of solutions containing both dsDNA and EG. The traditional graphical representation of a binding isotherm is obtained by plotting the fractional saturation binding (θ) of ligand to the receptor, calculated as a function of the free ligand concentration present in the solution at equilibrium. Assuming the fluorescence intensity is linearly proportional to the number of bound EG to the duplex DNA, the measured fluorescence intensity can be used[1–5] to calculate the fraction of EG bound to duplex DNA as a function of free equilibrium EG concentration ($[EG]_{eq}$). The fluorescence intensity as a function of $[EG]_{eq}$ is shown in Figure 1S for the solution containing poly-dA_{17}•poly-dT_{17} dsDNA and EG. The sigmoidal shape of the plot indicates cooperative binding of EG to dsDNA.

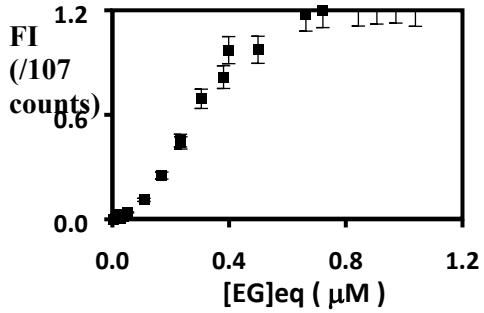

Figure 1S. Fluorescence intensity versus $[EG]_{eq}$ plot for intercalation of EG in 0.2 μM duplex poly-dA_{17}•poly-dT_{17} (10 mM Tris, 1 mM EDTA, 10 mM NaCl, pH 7.5). The data points represent the average (point) and standard deviation (error bars) from 4 separate experiments.

The fraction of EG bound or fractional saturation (θ) can be found from the fluorescence intensity as follows:

$$\theta = \frac{F_{obs} - F}{F_{max} - F} = \frac{concentration\ of\ bound\ EG}{concentration\ of\ binding\ sites} \quad (1)$$

Where F_{obs} and F are the observed fluorescence intensity at 532 nm of EG in the presence and the absence of 0.2 μM duplex DNA, respectively, and F_{max} is the maximum fluorescence attained at binding saturation. Figure 2S displays the binding isotherm obtained for the binding interaction of EG with duplex poly-dA_{17}•poly-dT_{17}.

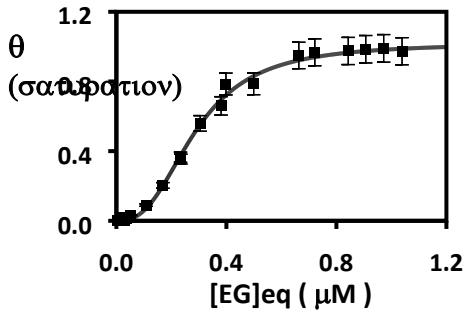

Figure 2S. Binding isotherm for the intercalation of EG into 0.2 μM duplex poly-dA_{17}•poly-dT_{17} (10 mM Tris, 1 mM EDTA, 10 mM NaCl, pH 7.5). The data points represent the average (point)

and standard deviation (error bars) from 4 separate experiments. The curve is the fit of the experimental data to the Hill equation (Eq. 5) using the parameters K_a^m = (3.5±0.0) x10^6 and m = 2.7±0.2.

To quantify the binding of EG to dsDNA, a simple equilibrium model first introduced by Hill and co-workers[6–11] was used as follows:

$$\text{DNA} + \text{mEG} \rightleftharpoons \text{DNA}\bullet\text{EG}_m \qquad (2)$$

$$K_d^m = \frac{[DNA]_{eq} \times [EG]_{eq}^m}{[DNA\bullet EG_m]_{eq}} \qquad (3)$$

In this model, the fractional saturation of the binding sites (θ) in duplex DNA available for occupation by the EG dyes is given by

$$\theta = \frac{m[DNA\bullet EG_m]_{eq}}{[DNA]_{eq,tot}} \qquad (4)$$

The fluorescence intensity from the dsDNA•EG_m complex is proportional to the number of bound EG, and $[DNA]_{eq,tot}$ refers to the dsDNA concentrations. In this model, duplex DNA is described as a system with a one-dimensional array of binding sites. Substitution of Equation 3 into Equation 4 and rearrangement yields the sigmoidal form of the Hill equation describing the ligand binding isotherm,

$$\theta = \frac{[EG]_{eq}^{m}}{K_d^m + [EG]_{eq}^{m}} \quad (5)$$

Figure 2S shows that Equation 5 fits the experimentally observed isotherm with m=2.7, indicating positive cooperativity for the intercalation binding of EG into duplex poly-dA_{17}•poly-dT_{17}. Further, Equation 5 can be easily rearranged to the well-known linear forms of the Hill equation,

$$\frac{\theta}{1-\theta} = \frac{[EG]_{eq}^{m}}{K_d{}^m} \quad (6)$$

$$log\frac{\theta}{1-\theta} = m\ log[EG]_{eq} - m\ logK_d \quad (7)$$

Although the Hill equation can be used to determine binding parameters such as the affinity constant and the number of ligands bound to duplex DNA, it rarely correctly describes the binding interaction; the Hill model, as presented in Eq. 7, is linear only for two limiting cases, completely non-cooperative binding when m = 1 and infinitely cooperative binding where the binding of the first ligand imparts infinitely high affinity for the subsequent ligands, i. e. the contributions of the intermediate complexes or partially occupied sites (DNA•EG_1, DNA•EG_2,……DNA•EG_{m-1}) are negligible and the system is dominantly in the form of either DNA or the fully-bound DNA•EG_m complex ("all-or-none" binding). Hence, the Hill model is useful only as a test for the deviation from a simple binding model and for detecting the presence of cooperativity (positive or negative) in the binding[6-11].

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