Electronic Supplementary Material

Influence of temperature and interactions with ligands on dissociation of dsDNA and ligand - dsDNA complexes of various type of binding. Electrochemical study

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Experimental

UV and CD methods

UV-Vis spectra were obtained with a Perkin Elmer spectrometer (model Lambda 25, Waltham, MA, USA), in a temperature range 5 - 100 °C with accuracy ± 0.5 °C. A special home - made thermostatic cell was applied in UV spectroscopic measurements. **Error! Bookmark not defined.** Temperature in the cell was additionally inspected with a thermometer.

In CD measurements a J-815 circular dichroism spectrometer (Jasco Inc, Easton, MD, USA), controlled by producer's software, was used. Temperature of the samples was controlled via a circular thermostat (Peltier, Traverse City, MI, USA). The measurements were done in the temperature range from 15 to 100 °C (accuracy ± 0.5 °C). The heating process and the stabilization of the temperature took usually 10 min. Before and during all experiments, dsDNA solutions were deoxygenated with pure nitrogen.

Results and discussion

UV-Vis measurements

***Intercalators*.** It is commonly known that the intercalation of drugs to the dsDNA is visualized as the hypochromic (absorbance decrease) or hyperchromic (absorbance increase) and bathochromic effects (shift of the maximum). It is seen in the insets in Figure 1SM that the maxima of absorbance of the studied intercalators alone in the solution are located at λ = 479 nm (EtBr), 406 nm (C-1305) and 419 nm (C-1311), while in the presence of dsDNA they are shifted towards shorter wavelength by circa 50 nm (EtBr), 10 nm (C-1311) and 3 nm (C-1305).

We have also observed the changes in the position and height for the band characteristic for dsDNA (placed at λ=260 nm). The DNA spectra obtained at various temperatures are included in Figure 1SM. In the absence of dsDNA, the absorbance of investigated intercalator-DNA complexes placed at λ=260 nm increased with an increase in temperature. The determined melting points of the double strand equaled 60, 84, 86 and 82 ºC for unbound with ligands DNA and its complexes with EtBr, C-1305 and C-1311, respectively. This is illustrated in Figure 2SM. Apparently, for unbound dsDNA the melting point is substantially lower, by circa 20 % compared to those of the dsDNA-drug complexes. By examining the

appropriately normalized absorbance for the wavelength characteristic only for the given drug it can be monitored how the drug is released or partially liberated from the complex. The obtained dependencies are shown in the insets of Figure 2SM. They lead to the following conclusions: the process of separating EtBr from the DNA strand starts after passing the melting point: i.e. at temperatures higher than 80 ºC. Contrary to EtBr the start of releasing or partial releasing of C-1305 and C-1311 from the dsDNA-drug complexes precedes a little the unwinding process. It's worth noting that only at 100 ºC the plots of relative absorbancies meet, which means that all examined drugs, are now fully separated. The complex shape of the C-1311 and C-1305 dependencies in the dsDNA inset reflects the competition between the dimerization of the ligands (see the free-ligands inset in Figure 2SM) and the intercalation of dsDNA.

Groove binder. Bis-benzimidazole (Hoechst 33258) preferentially binds at the minor grove of A-T rich sequences of dsDNA. A set of absorbance spectra of the dsDNA-drug mixtures and of the free components obtained at various temperatures (10 ºC - 100 ºC) is presented in Figure 3A-SM. The interaction of Hoechst 33258 with dsDNA duplex leads, similarly to the examined intercalators, to the hypochromic and batochromic effects. With an increase in temperature the absorbance at the band characteristic for Hoechst 33258 - dsDNA significantly increases and that band shifts to less positive wavelength characteristic for the free species. Apparently the complex with Hoechst 33258 dissociates completely since the plots meet at 100 ^{o}C. The way the minor - grove binder, Hoechst 33258, gradually leaves the dsDNA helix with increasing temperature is shown in the inset of Figure 3B-SM. Hoechst is released partially in the relatively low temperature range (35 ÷ 45 °C) and is finally fully liberated at over 90 °C. The denaturation process of complexed dsDNA starts at a much higher temperature, by 23 °C, in comparison to that of dsDNA unbound.

Covalent binder. *Cis*-Pt does interact with dsDNA since the absorbance of the mixture of the drug with dsDNA is higher than the sum of absorbances of free components. This is illustrated in Figures 4A-SM. In contrast to the compounds described above, the absorbance of *cis*-Pt alone is negligible. In the presence of *cis*-Pt the dsDNA melting temperature is practically unchanged ($T_m \approx 60$ °C), see Figure 4B-SM. Figure 4B-SM also indicates that *cis*-Pt is not fully liberated even after the total dissociation of the DNA double strand: since the lines in the plots do not meet.

CD measurements

It is known, that CD spectrum of dsDNA in right-handed helix B conformation exhibits two bands at the UV region: a negative band with minimum at 246 nm (hydrogen bonds) and a positive one with maximum at 276 nm (stacking bonds). The conformation changes of the helix, induced by the drug-DNA interactions or the increase in temperature, are reflected in its CD spectrum.

Intercalators. The CD spectra of dsDNA obtained in the presence of the acridinones and EtBr is from 10 °C to 100 ºC are presented in Figures 5SM. The common thing for C-1311 and C-1305 is a big decrease in the ellipticity within the negative band and a small decrease within the positive band of dsDNA. Contrary to acridinones the ellipticity for unbound dsDNA within the negative band of EtBr grows substantially. Also a well-defined, positive inductive band appears for EtBr - dsDNA complex.

As temperature increases, for both acridinones, the changes in ellipticity resemble those of the absorbance; substantial changes appear at circa 70 °C (see the insets in Figure 5SM). Again, contrary to

this, ellipticity (measured for EtBr) changes rather continually with temperature (insets in Figure 5SM. Since, compared to EtBr, the acridinines have got “tails”, it can be concluded that those tails stabilize additionally the intercalating interactions. This conclusion is confirmed by the values of the melting temperatures obtained from UV-Vis spectroscopy measurements. They are substantially higher (see Table 1 in the manuscript).

Groove binder. The circular dichroism spectra for the complex of Hoechst 33258 with dsDNA in a physiological solution (PBS, pH = 7.4) for various temperatures (10 °C - 100 °C) are presented in Figure 6SM. At room temperature the interactions between Hoechst 33258 and dsDNA cause a significant decrease in dsDNA ellipticity at the negative band as well as the positive one. Similarly to intercalator EtBr, this compound also exhibits an induced band (after binding to dsDNA) with a maximum at 354 nm. As temperature increases, for both bands, the dsDNA ellipticity only slightly increases. A shift of the negative band by circa 5 nm towards higher wavelengths was observed, whereas the positive band maximum shifted by bands, the dsDNA ellipticity only slightly increases. A shift of the negative band by circa 5 nm towards higher wavelengths was observed, whereas the positive band maximum shifted by circa 10 nm towards lower wavelength. Ellipticity measured for the induced band strongly, linearly decreased, down to 0, with increasing temperature. We interpret the latter strong change in terms of stepwise detachment of the drug from the dsDNA strand, which is in agreement with the UV-Vis spectroscopic data in the inset of Figure 3B-SM.

Covalent binder. Typical CD spectra obtained for the *cis*-Pt and dsDNA complex in different temperatures are presented in Figure 7SM. The value of ellipticity of the negative band increased and was shifted circa 5 nm towards higher values of wavelength after increasing temperature. Insets in Figure 7SM correlate well with the absorbance plots in Figure 4B-SM, which supports the conclusion that no substantial changes in the value of the melting temperature of dsDNA occur in the presence of *cis*-Pt.

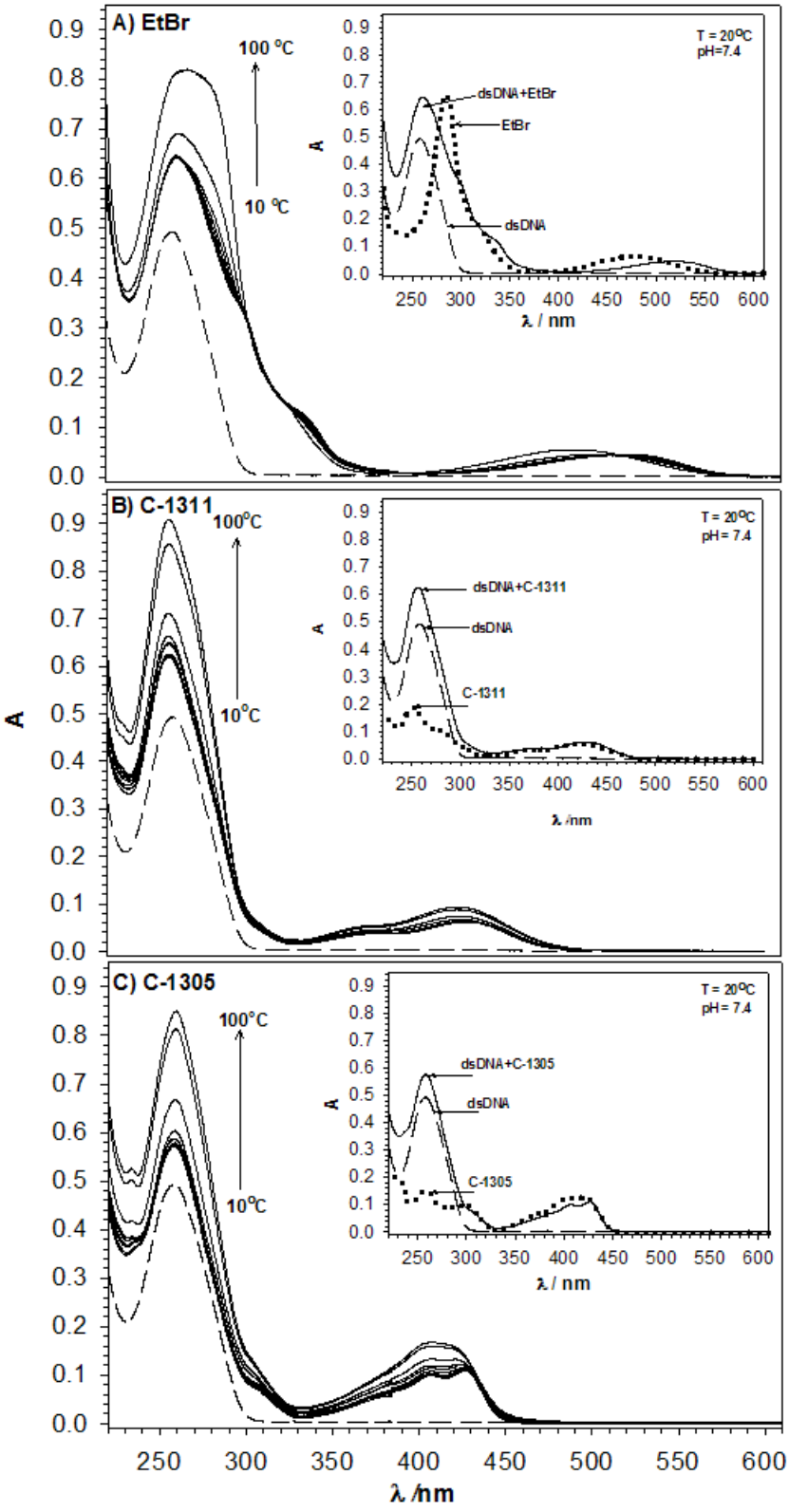

Figure 1SM.
Spectrograms (absorbance vs. wavelength) of 38.2 μM base pairs dsDNA (dashed line), 0.01 mM unbound intercalators (dotted lines), and mixtures of dsDNA and intercalators (solid lines). 0.01 M PBS (pH = 7.4), temperature range 10 ºC - 100 ºC. Intercalators: EtBr (A), C-1311 (B), and C-1305 (C). Insets: dependencies of absorbance obtained at 20 ºC vs. wavelength for dsDNA, unbound compounds, and their mixtures with dsDNA.

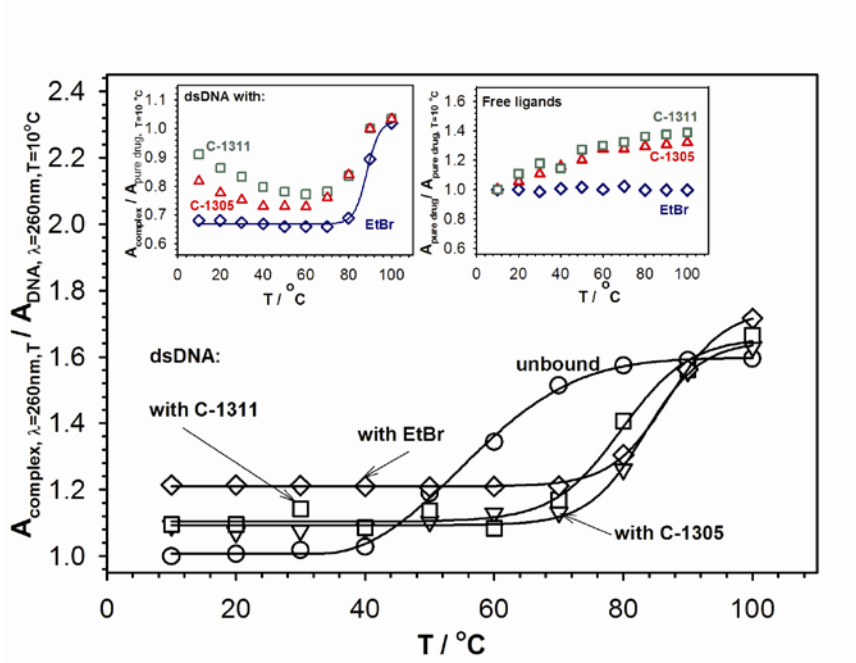

Figure 2SM.
Plots of normalized maximal absorbance (at λ = 260 nm) vs. temperature for denaturation processes of dsDNA unbound and dsDNA in the presence of C-1311, C-1305 and EtBr. Insets: normalized absorbance of dsDNA-drug mixtures vs. temperature measured at wavelength specific for drugs. Conditions as in Figure 1.

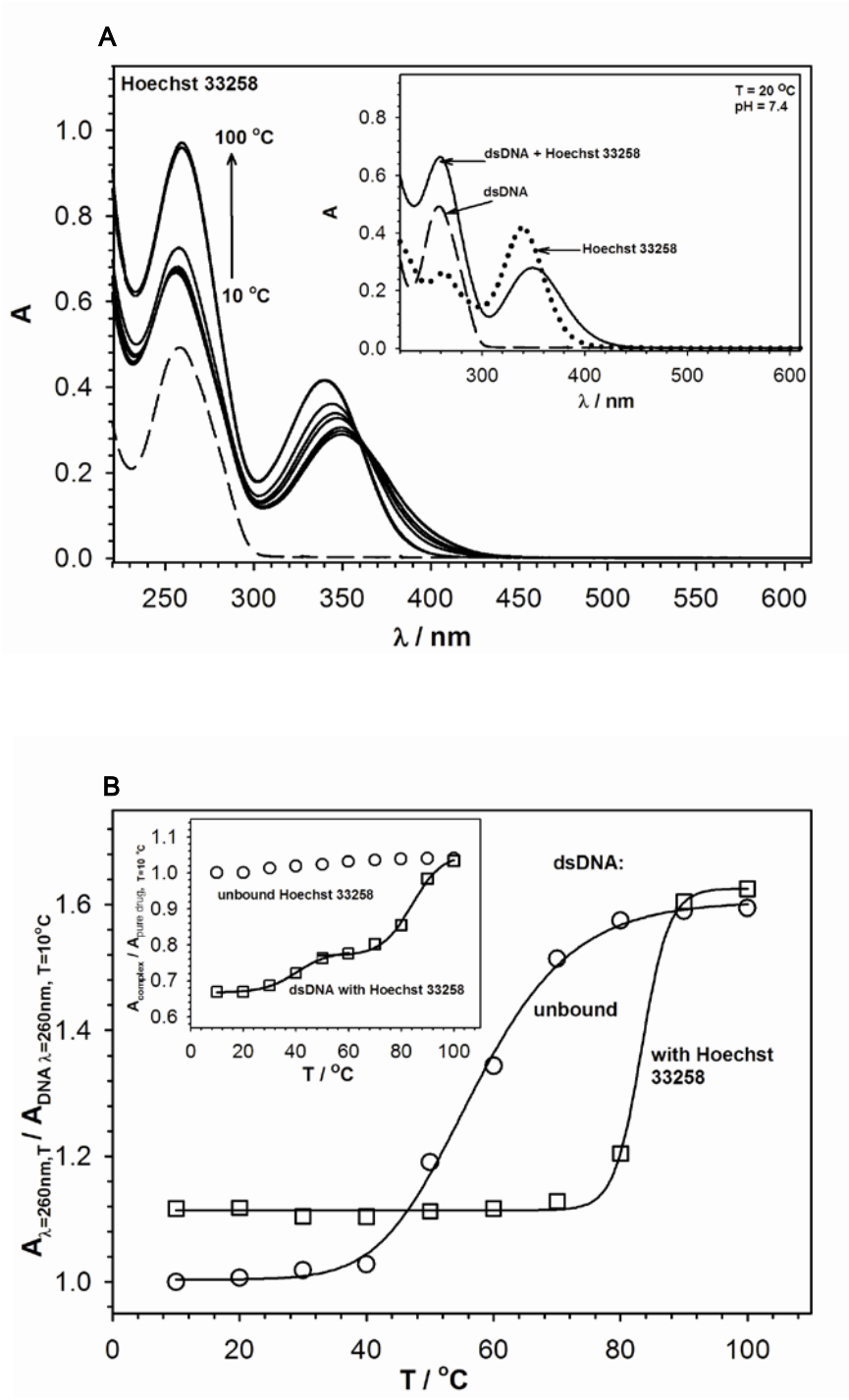

Figure 3SM.

A) Spectrograms (absorbance vs. wavelength) of 38.2 μM base pairs dsDNA (dashed line), unbound 0.01 mM Hoechst 33258 (dotted line) and its mixtures with dsDNA (sold line). 0.01 M PBS, temperature range 10 ºC - 100 ºC.

B) Dependencies of normalized absorbance (measured at wavelength specific for dsDNA) vs. temperature for denaturation process of unbound dsDNA and in presence of Hoechst 33258. Inset: plots of normalized (vs. free drug at 10 ºC) absorbance (measured for each temperature at wavelength of maximum absorbance specific for drug) vs. temperature for unbound Hoechst 33258 and in presence of dsDNA.

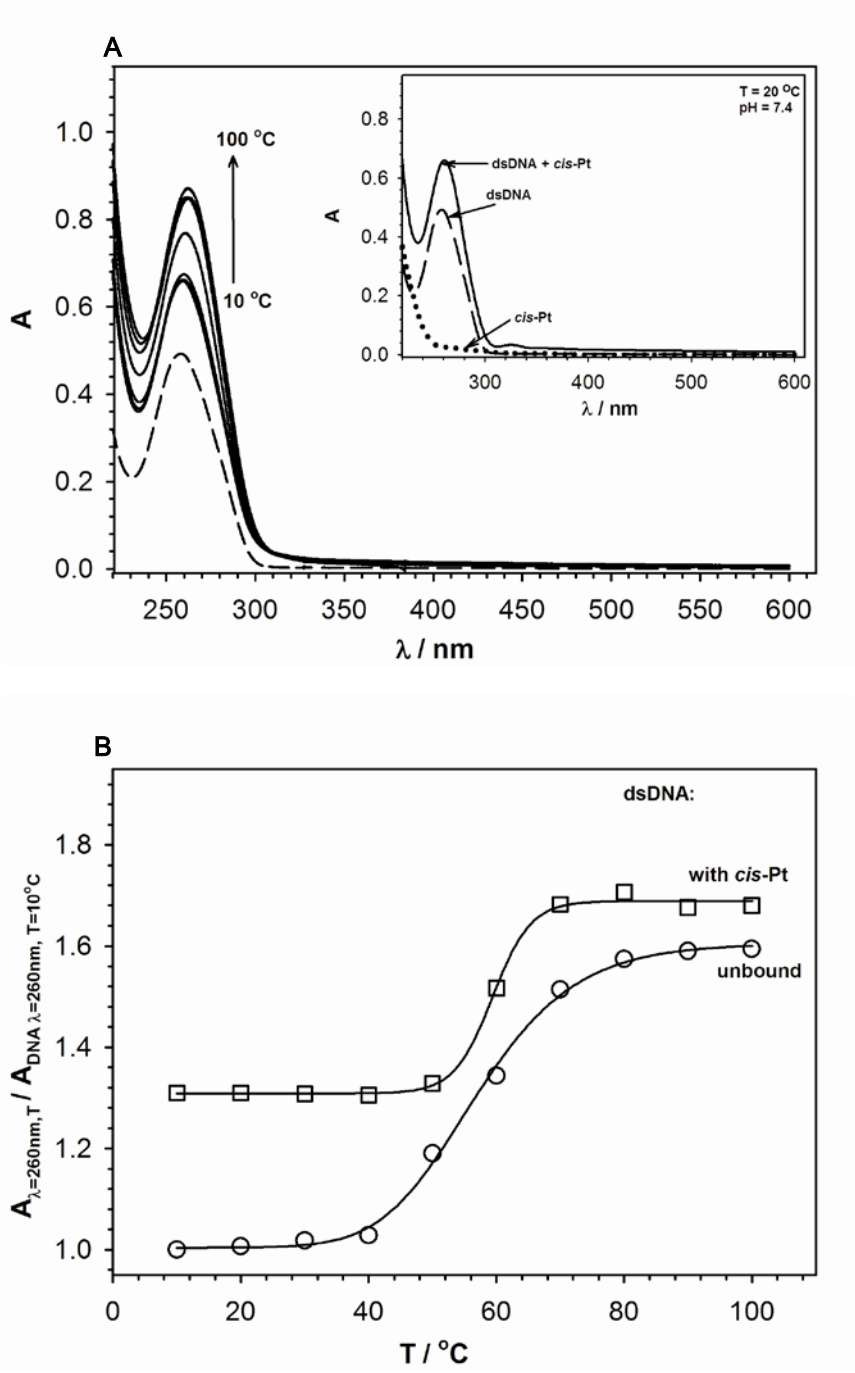

Figure 4SM.

A) Absorbance vs. wavelength for 38.2 μM base pairs dsDNA (dashed line), 0.01 mM *cis*-Pt (dotted line) and its mixtures with dsDNA (solid line), in 0.01 M PBS; temperature range 10 ºC - 100 ºC. Inset: dependencies of absorbance vs. wavelength for dsDNA obtained at 20 ºC for free compounds, and their mixtures with dsDNA

B) Dependencies of normalized (vs. T = 10 ºC) absorbance measured at λ = 260 nm vs. temperature for denaturation process of unbound dsDNA and in presence of *cis*-Pt.

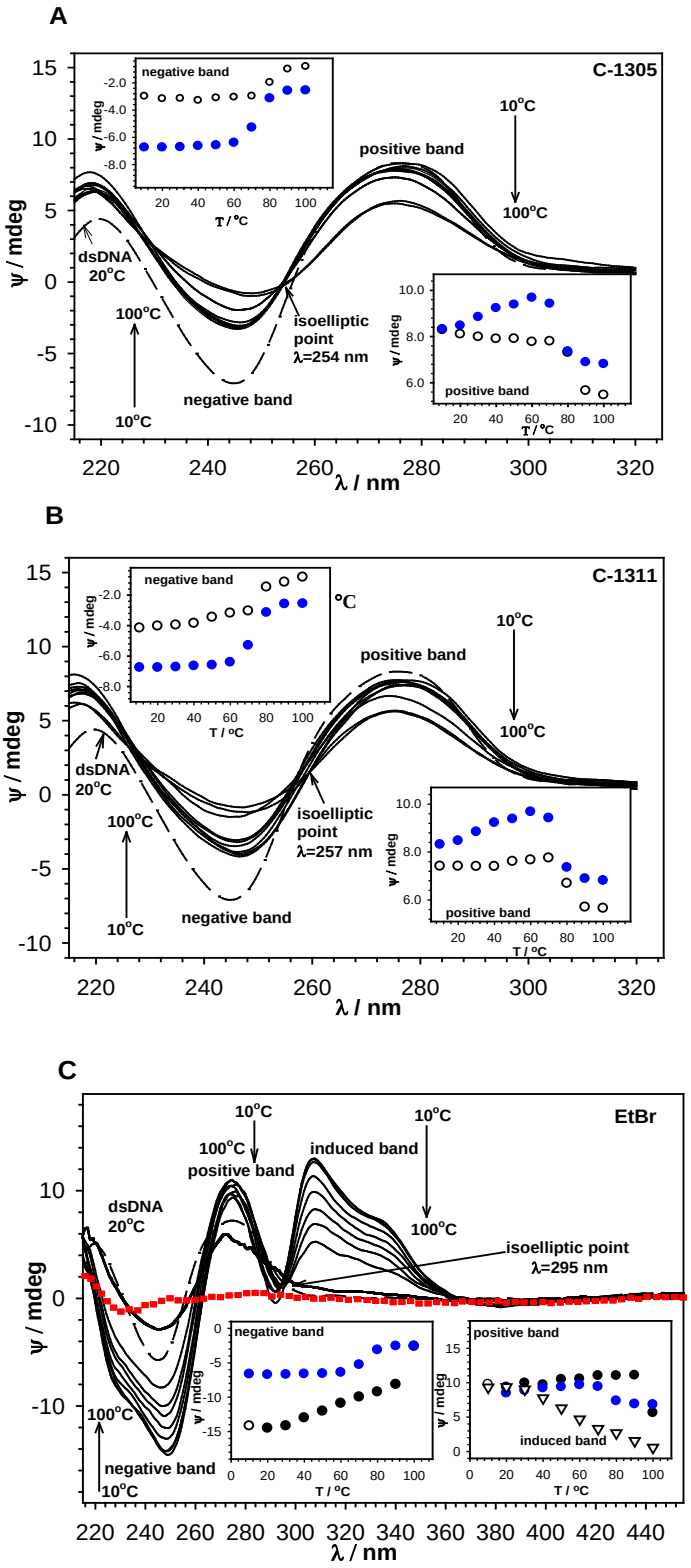

Figure 5SM.
CD spectra (ellipticity vs. wavelength) for 38.2 μM base pairs dsDNA mixtures with 0.01 mM C-1305 (A), C-1311 (B) and EtBr (C) obtained at various temperatures. Dashed line: unbound dsDNA, 20 ºC, dotted line unbound EtBr. Insets: Plots of changes of ellipticity vs. temperature obtained in minimum and maximum of negative (circles) and positive (diamonds) bands and in induced (triangle down) band of CD spectra, respectively, in the temperature range 10 – 100 ºC. Experimental conditions as in Figure 1.

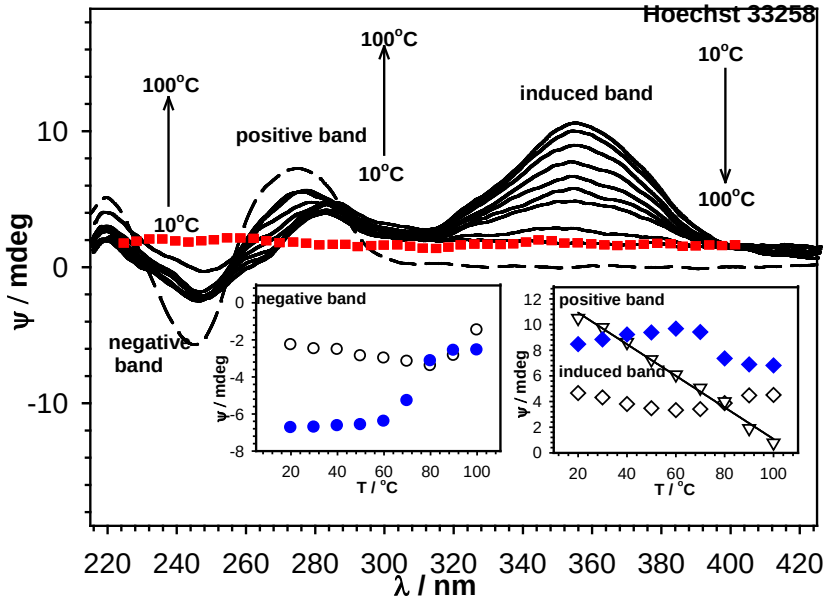

Figure 6SM
CD spectra (ellipticity vs. wavelength) of 38.2 μM dsDNA base pairs in presence of 0.01 mM Hoechst 33258. 0.01 M PBS, various temperatures. Dashed line: dsDNA, 20 ºC, dotted line: unbound Hoechst 33258, 20 ºC. Insets: Plots of changes of ellipticity vs. temperature obtained in negative (circles), positive (diamonds) and induced (triangle down) bands of dsDNA/Hoechst 33258. Hollow circles: complex intercalator – DNA, filled circles: unbound DNA.

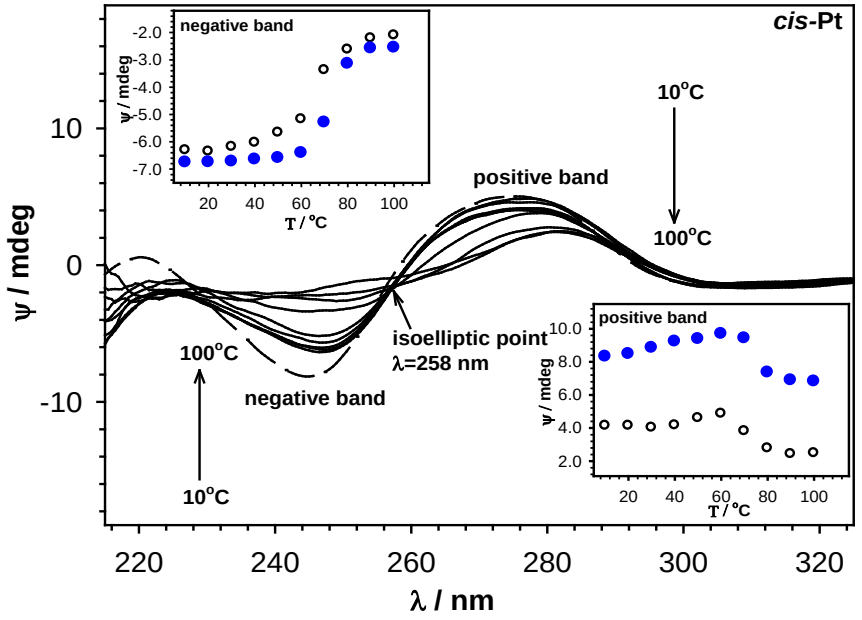

Figure 7SM
CD spectra (ellipticity vs. wavelength) of 38.2 μM dsDNA base pairs in presence of 0.01 mM *cis*-Pt in 0.01 M PBS and at various temperatures. Dashed line, unbound dsDNA, 20 ºC. Insets: Plots of changes of ellipticity vs. temperature measured in negative and positive bands of dsDNA/*cis*-Pt spectra. Filled circles stand for changes of ellipticity obtained for dissolved, unbound dsDNA, open circles stand for changes of ψ vs. T for dsDNA in presence of *cis*-Pt.