Supporting Information

Preferential Binding of Epirubicin Hydrochloride with Single Nucleotide Mismatched DNA and Subsequent Sequestration by a Mixed Micelle

Laxmikanta Khamari, Srikrishna Pramanik, Shashi Shekhar, Paritosh Mahato, and Saptarshi Mukherjee*

Department of Chemistry

Indian Institute of Science Education and Research Bhopal

Bhopal Bypass Road, Bhauri, Bhopal 462066, Madhya Pradesh, India

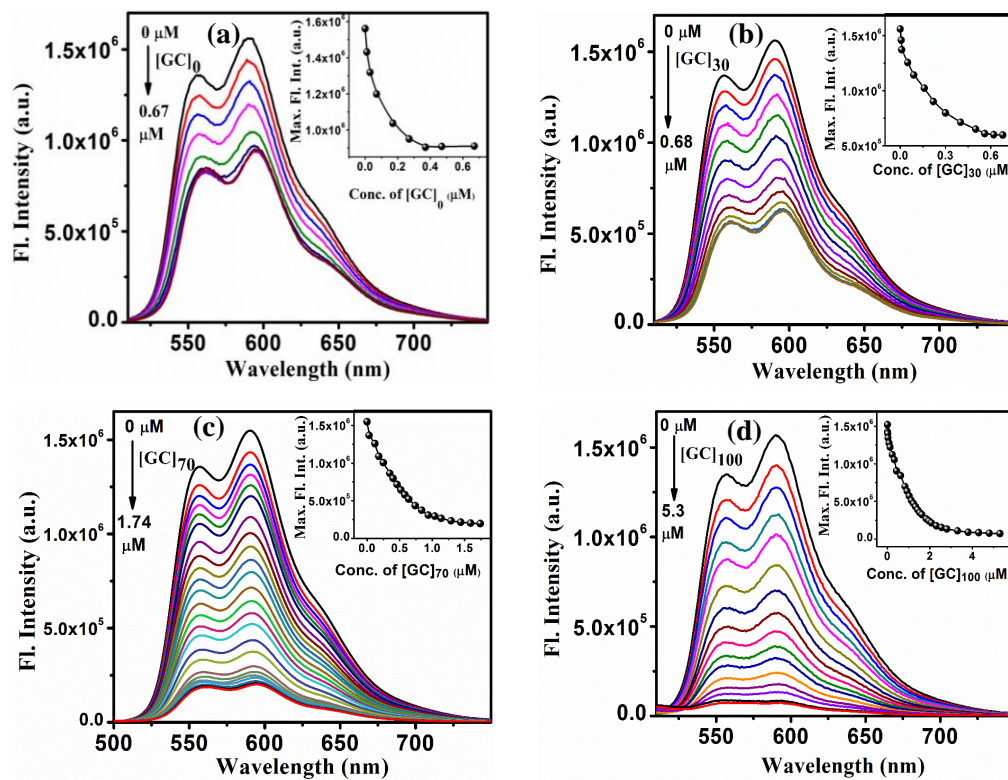**Corresponding author: E-mail: saptarshi@iiserb.ac.in, Tel.: +91-755-269-1301.*

Figure S1: Emission profiles of EPR (3 µM) in the absence and presence of variant concentrations of ds DNA for (a) [GC]$_0$ DNA, (b) [GC]$_{30}$ DNA, (c) [GC]$_{70}$ DNA, and (d) [GC]$_{100}$ DNA.
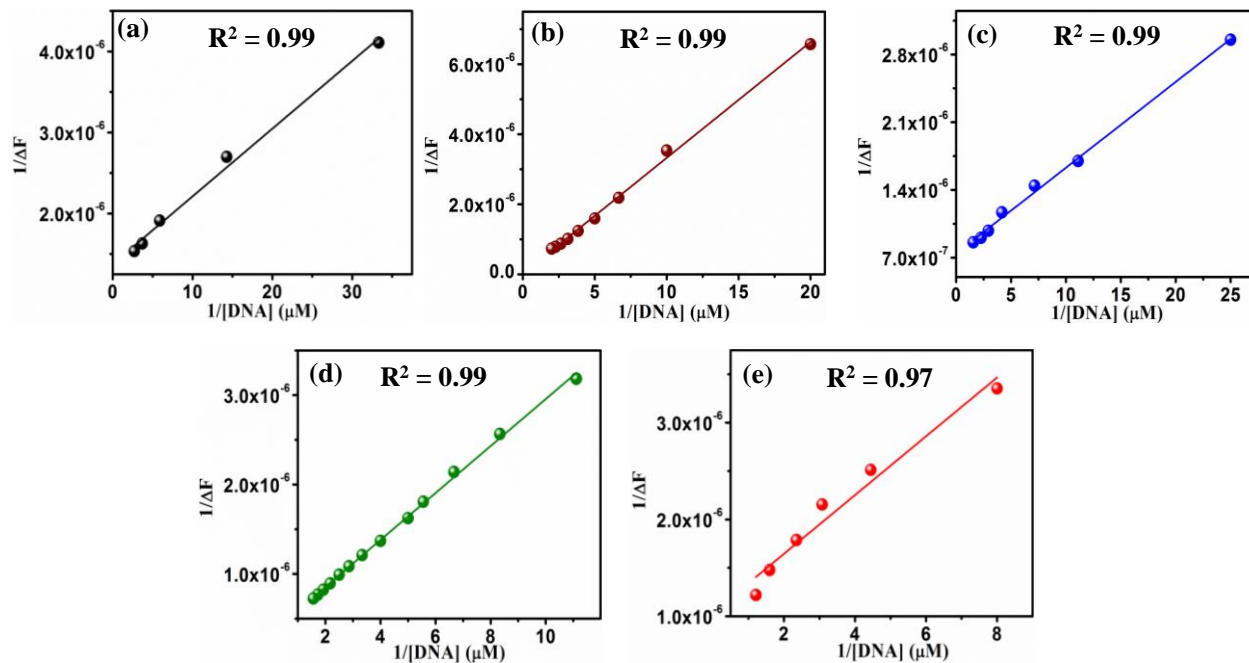
Figure S2: Benesi-Hildebrand plots of the EPR-DNA complex for (a) $[GC]_0$, (b) $[GC]_{30}$, (c) $[GC]_{50}$ or WM, (d) $[GC]_{70}$, and (e) $[GC]_{100}$ DNA.
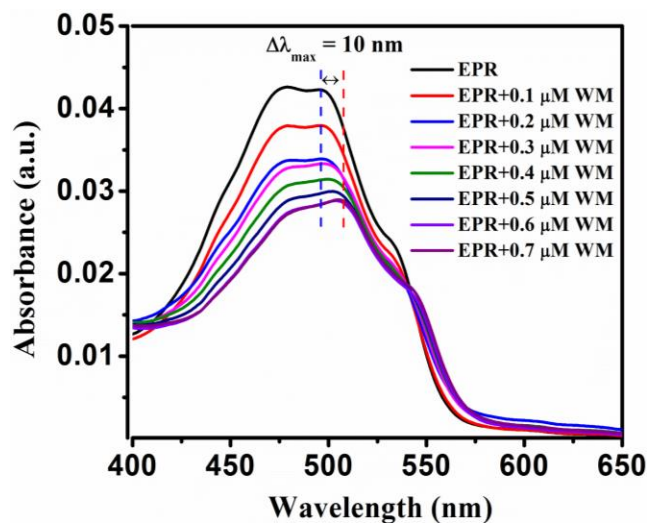
Figure S3: Absorption spectra of EPR (3 µM) in the absence and presence of ds WM DNA (with varying concentrations) having 50 % AT/GC base pair composition.
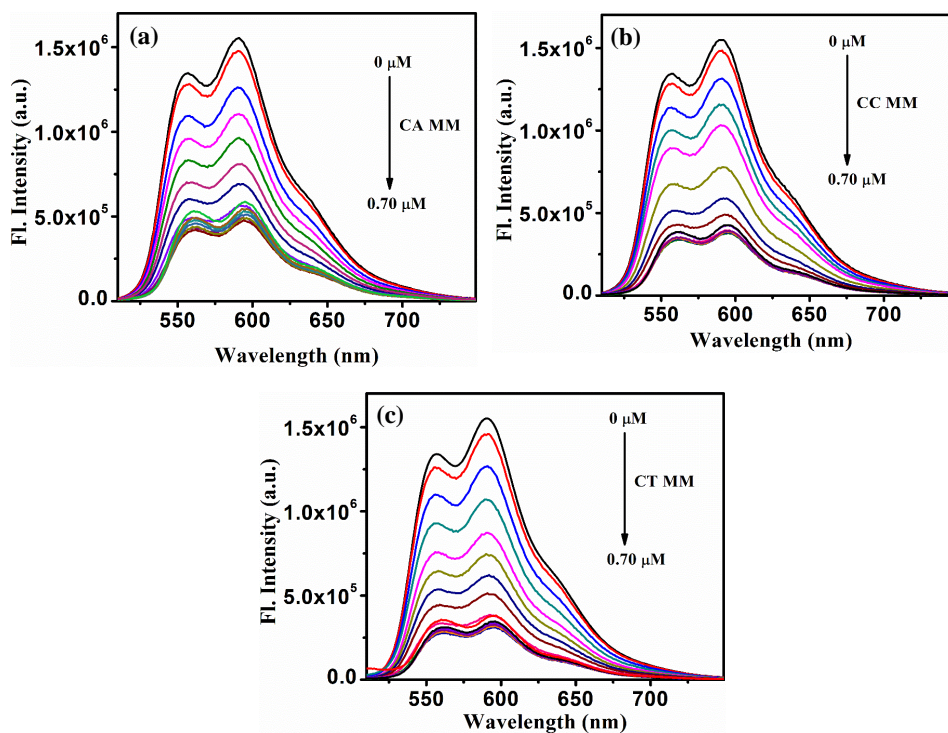

Figure S4: Emission profiles of EPR (3 µM) in the absence and presence of ds DNA (varying concentrations from 0 to 0.70 µM) for (a) CA MM DNA, (b) CC MM DNA, and (c) CT MM DNA.
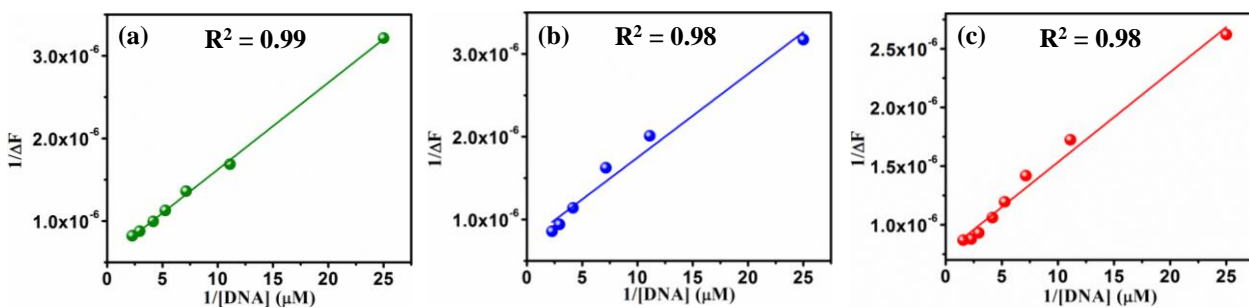
Figure S5: Benesi-Hildebrand plots of EPR-MM DNA complexes for (a) CA MM, (b) CC MM and (d) CT MM DNA.
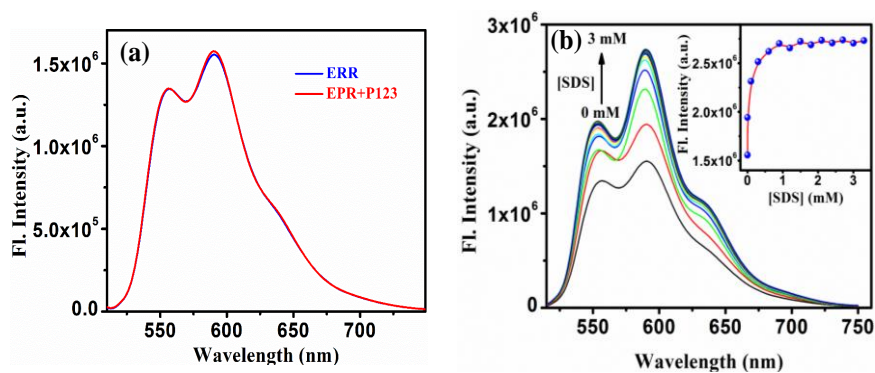

Figure S6: (a) Fluorescence spectra of EPR (3 µM) with and without 0.5 mM P123. (b) Emission spectra of EPR (3 µM) containing 0.5 mM P123 in the absence and presence of variant concentrations of SDS as marked in the figure. The inset represents the variation of fluorescence peak intensities of EPR as a function of [SDS].
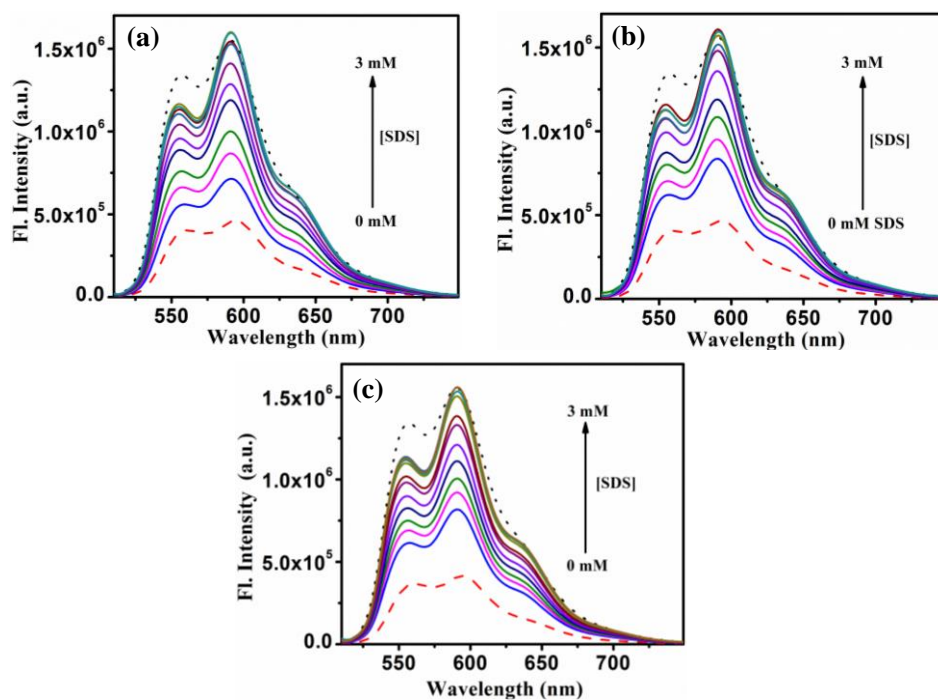
Figure S7: Emission profiles of EPR (3 µM) in EPR-DNA complex containing P123 (0.5 mM) in the absence and presence of variant concentrations of added SDS for (a) CA MM, (b) CC MM, and (c) CT MM DNA.
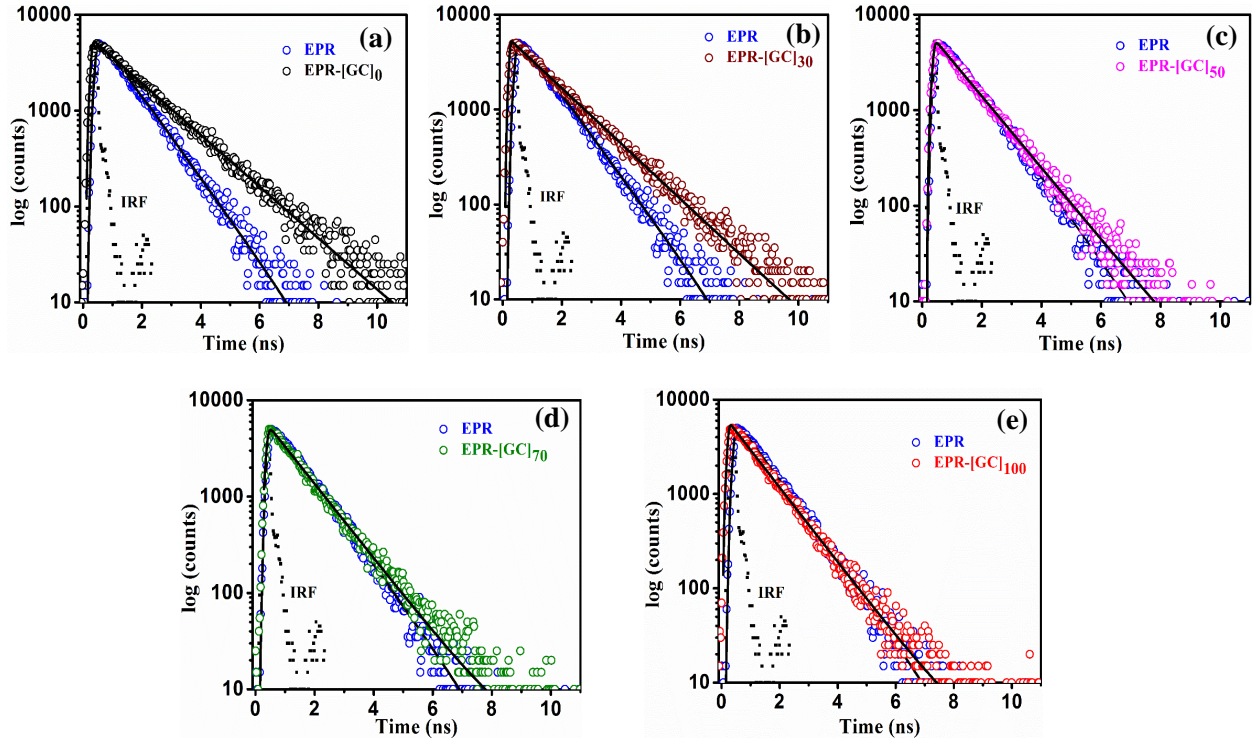
Figure S8: Fluorescence lifetimes of EPR (3 μM) in the absence and presence of ds DNA for (a) $[GC]_0$ (0.37 μM), (b) $[GC]_{30}$ (0.56 μM), (c) $[GC]_{50}$ or WM (0.70 μM), (d) $[GC]_{70}$ (1.60 μM), and (e) $[GC]_{100}$ (5.3 μM) DNA.

Table S1: Fluorescence Lifetimes Parameters of EPR in the Absence and Presence of ds DNA Having Different Compositions of GC Contents

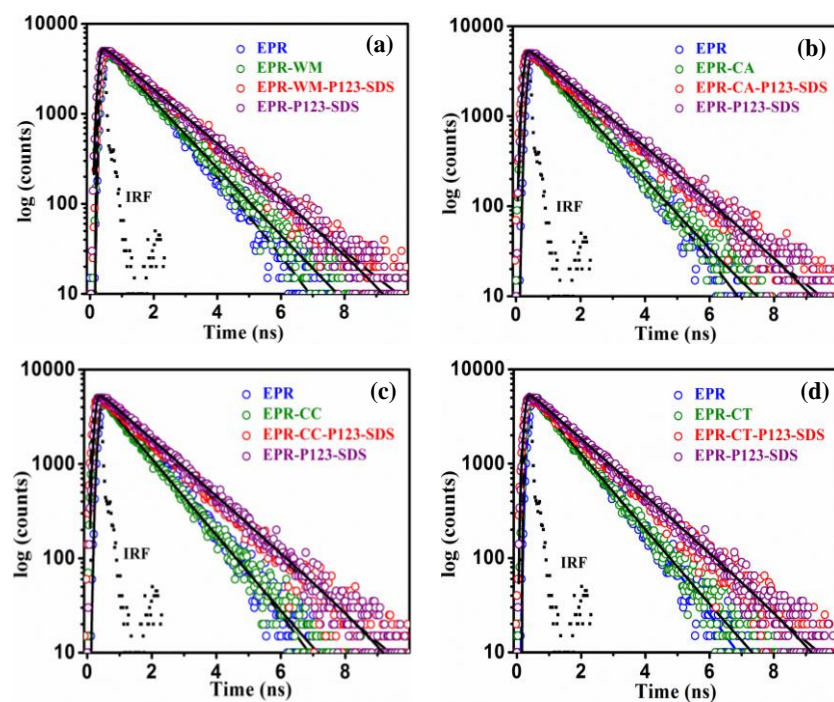| system	α	τ^a (ns)	χ^2
EPR	1.00	1.01	1.08
EPR-$[GC]_0$	1.00	1.60	1.01
EPR-$[GC]_{30}$	1.00	1.49	1.01
EPR-$[GC]_{50}$	1.00	1.16	1.00
EPR-$[GC]_{70}$	1.00	1.12	1.00
EPR-$[GC]_{100}$	1.00	1.09	1.02

[a] $\pm$ 5%

Figure S9: Fluorescence lifetimes of EPR and EPR-P123-SDS in the absence and presence of ds DNA for (a) WM, (b) CA MM, (c) CC MM, and (d) CT MM DNA.

Table S2: Fluorescence Lifetime Decay Parameters of EPR and EPR-P123-SDS in the Absence and Presence of Different WM/MM DNAs

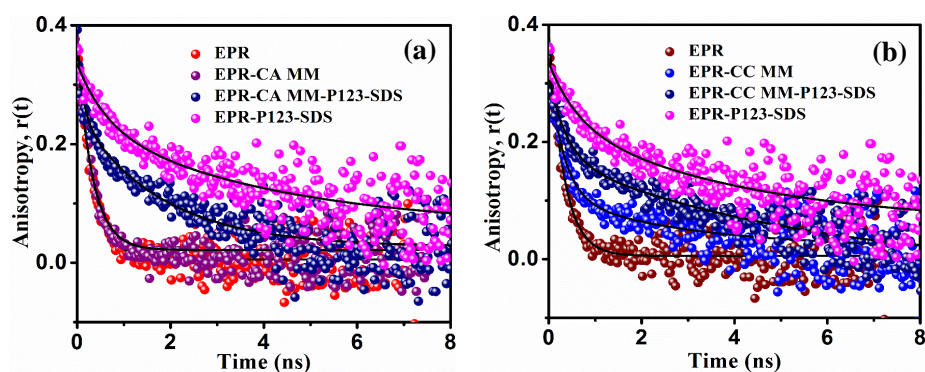| System	α	τ^a (ns)	χ^2
EPR	1.00	1.01	1.08
EPR-P123-SDS	1.00	1.44	1.00
EPR-WM DNA	1.00	1.16	1.00
EPR-WM-P123-SDS	1.00	1.44	1.00
EPR-CA MM DNA	1.00	1.10	1.00
EPR-CA MM-P123-SDS	1.00	1.44	1.00
EPR-CC MM DNA	1.00	1.10	1.00
EPR-CC MM-P123-SDS	1.00	1.44	1.00
EPR-CT MM DNA	1.00	1.10	1.01
EPR-CT MM-P123-SDS	1.00	1.44	1.01

[a] $\pm$ 5%

Figure S10: Fluorescence anisotropy decay profiles of only EPR and EPR-P123-SDS in the absence and presence of (a) CA, and (b) CC MM DNA.
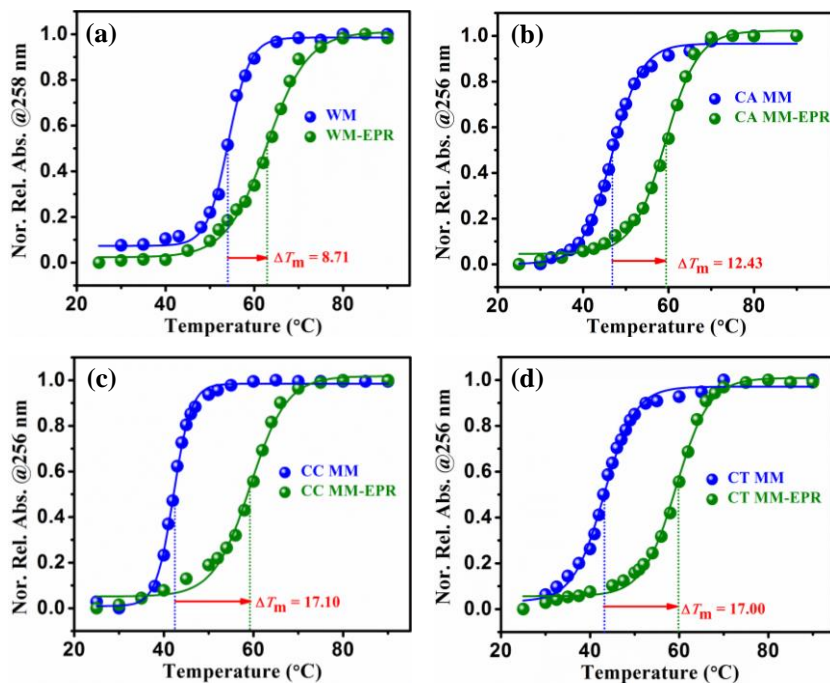

Figure S11: Melting curves of (a) WM DNA, (b) CA MM, (c) CC MM and (d) CT MM without EPR and with EPR (3 μM). For all the melting experiments, DNA concentrations were kept at 0.7 μM.
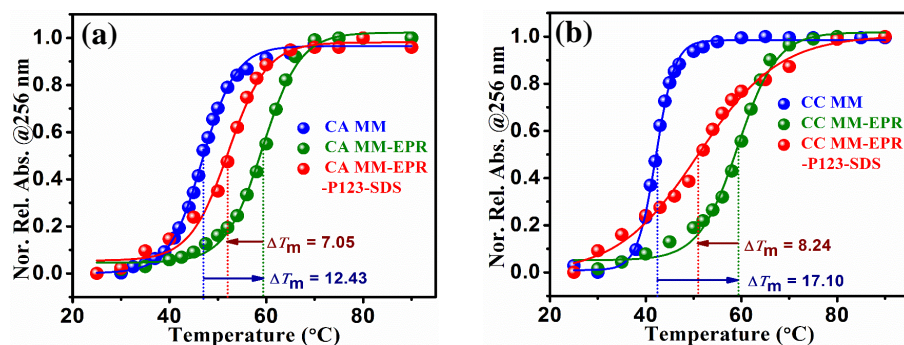
Figure S12: Melting curves of (a) CA MM, and (b) CC MM in the free form, in the presence of 3 µM EPR and the presence of both EPR and mixed micelle (P123-SDS) as marked by the color codes in the figures. For all the melting experiments, DNA concentrations were kept at 0.7 µM.

Figure S13: (a) Fluorescence emission spectra of EPR (3 µM) in the presence of different concentrations of GG MM DNA (5′-ATATATATATG**G**GCGCGCGC-3′/3′-TATATATATAC**G**CGCGCGCG-5′). (b) Benesi-Hildebrand plots of EPR-GG MM DNA complex. (c) Melting curves of GG MM DNA in the absence and presence of EPR (3 µM) and in the presence of mixed micelle (P123-SDS) as marked by the color codes in the figure. For all the melting experiments, DNA concentrations were kept at 0.7 µM.

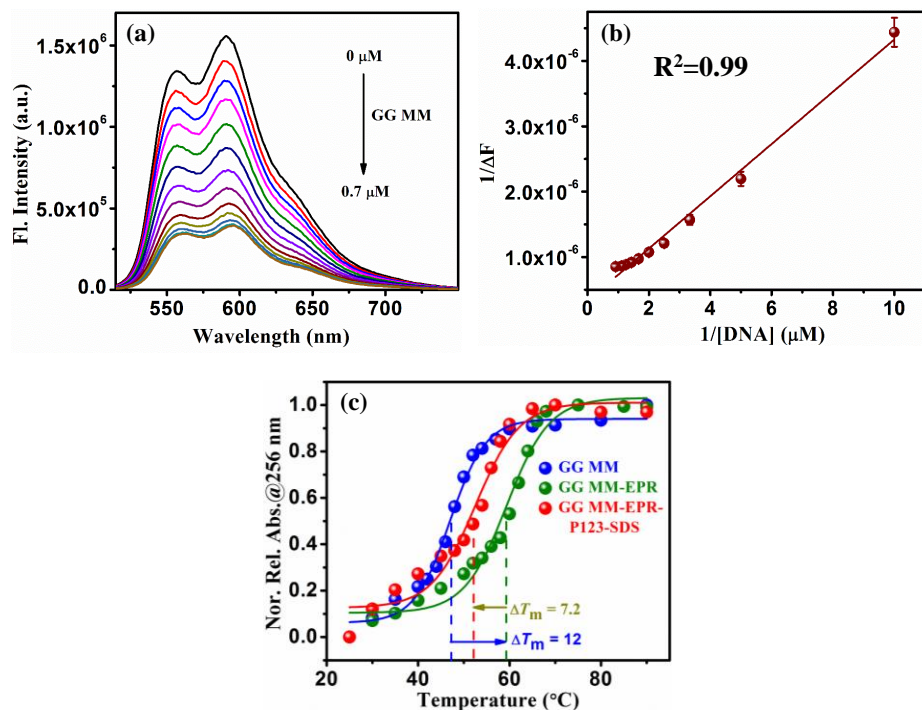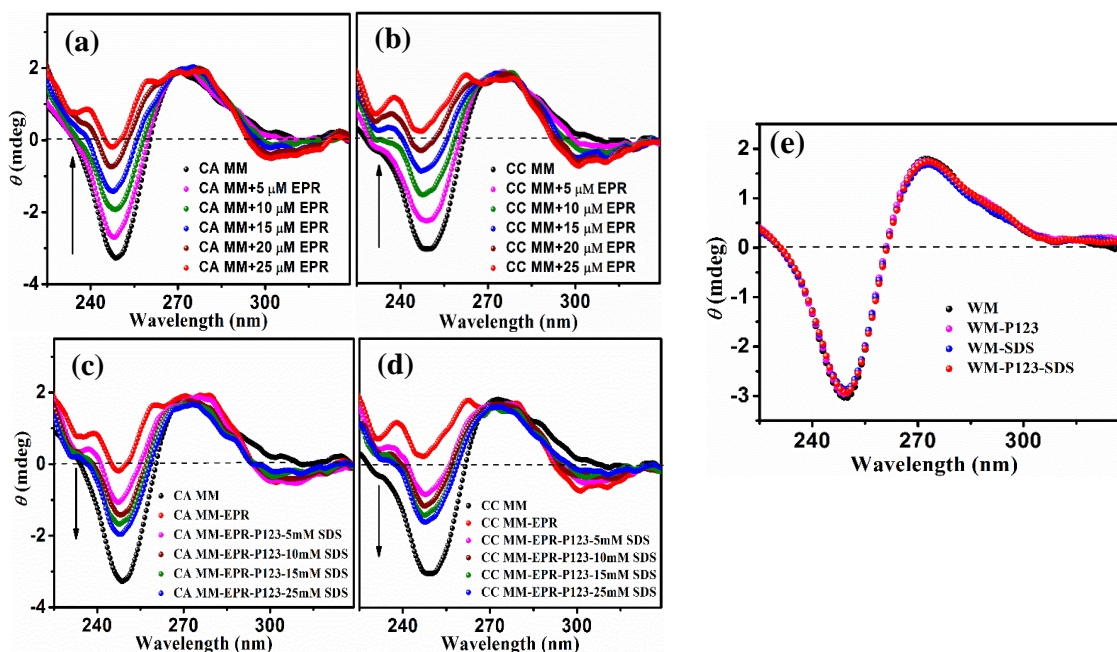**Figure S14**: CD spectra of (a) CA MM and (b) CC MM DNA in the absence and presence of different concentrations of EPR (0 to 25 μM) as marked in the figures. The release of EPR from EPR-DNA complex containing a fixed concentration of P123 co-polymer and varying concentrations of SDS as marked in the figures; for CA MM DNA (c), and CC MM DNA (d). (e) CD bands of WM DNA do not alter in the presence of P123, SDS, and P123-SDS system (SDS: P123 = 6).
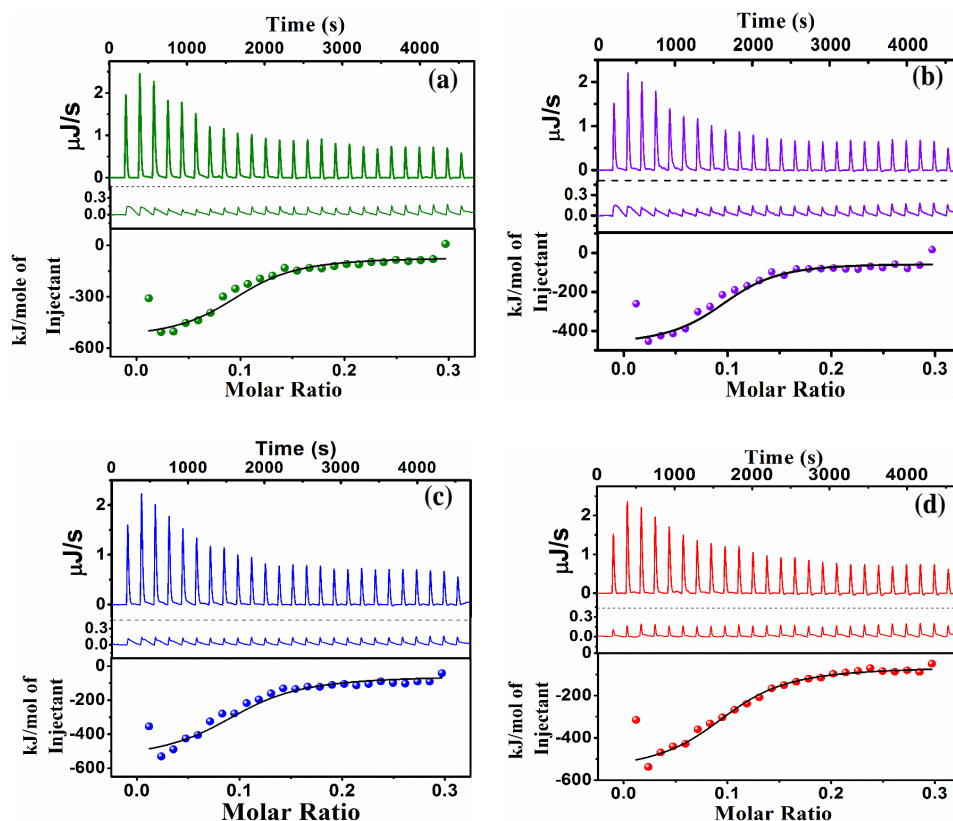
Figure S15: ITC titration profiles of the interaction of EPR with WM DNA (a), CA MM DNA (b), CC MM DNA (c), and CT MM DNA (d). The upper, middle, and lower panels of all the plots (a, b, c and d) respectively, represent the raw heat change data (after correction of the heat of dilution), only heat of dilution of titrant (here, DNA), and ITC enthalpograms of the said interaction.

Table S3: Associated Thermodynamic Parameters for the Binding of EPR to Various DNA Systems Obtained from ITC Experiments

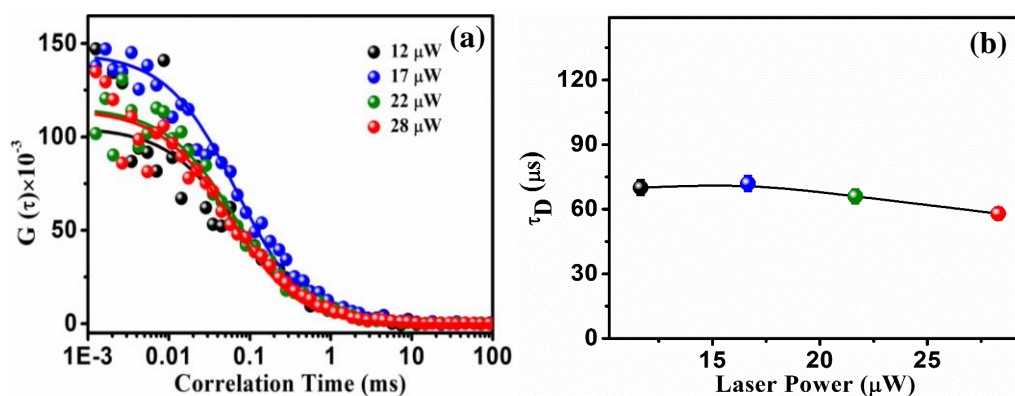| system	K_a (M^{-1})	n	ΔH (kJ/mol)	$T\Delta S$ (kJ)	ΔG (kJ/mol)
WM DNA	$1.60\pm0.02 \times 10^6$	0.12 ± 0.002	-99.03±5	-63.27±2	-35.76
CA MM DNA	$2.10\pm0.05 \times 10^6$	0.12 ± 0.003	-100.05±3	-63.80±3	-36.25
CC MM DNA	$10.01\pm0.04 \times 10^6$	0.11 ± 0.001	-102.10±4	-64.37±2	-37.73
CT MM DNA	$10.20\pm0.05 \times 10^6$	0.13 ± 0.003	-103.00±3	-64.22±4	-38.78

Figure S16: (a) FCS traces of EPR (10 nM) at different incident laser powers as marked in the figure. (b) Diffusion times (τ_D in μs) of EPR at different laser powers as marked in the figure.
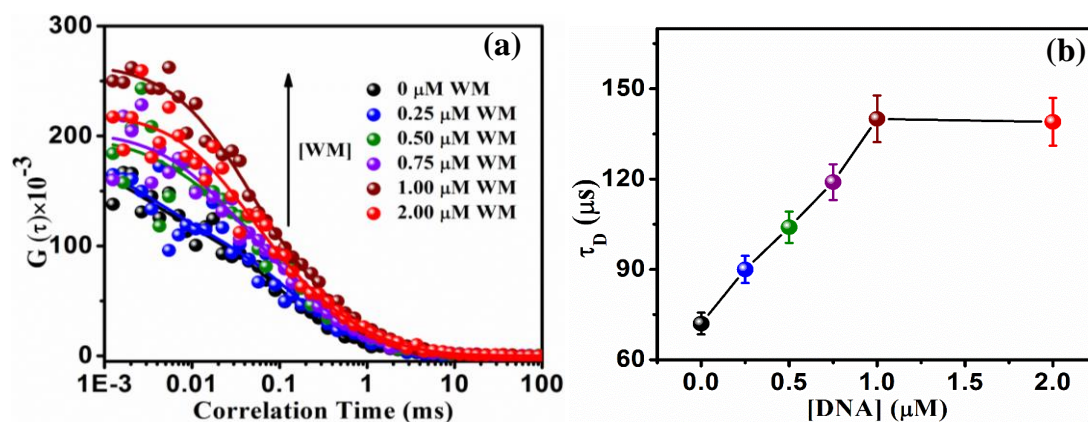

Figure S17: (a) FCS traces of EPR (10 nM) at various concentrations of WM DNA. (b) Variation of diffusion times (τ_D in μs) of EPR at different concentrations of WM DNA.

Table S4: τ_{off} and τ_{on} Parameters of EPR-DNA Systems Obtained from Fluorescence Intensity Fluctuations

system	τ_{off} (μs)			τ_{on} (μs)		
	τ_{off1} (α_1)	τ_{off2} (α_2)	$<\tau_{off}>$ (μs)	τ_{on1} (α_1)	τ_{on2} (α_2)	$<\tau_{on}>$ (μs)
WM DNA	60 (0.41)	1.2 (0.59)	25.3	0.13 (0.54)	0.13 (0.46)	0.13
CA MM DNA	67 (0.20)	1.1 (0.80)	14.3	0.11 (1.0)	-	0.11
CC MM DNA	21 (0.56)	1.1 (0.44)	12.2	0.11 (0.99)	0.26 (0.01)	0.11
CT MM DNA	17 (0.62)	1.0 (0.38)	10.9	0.14 (0.53)	0.14 (0.47)	0.14