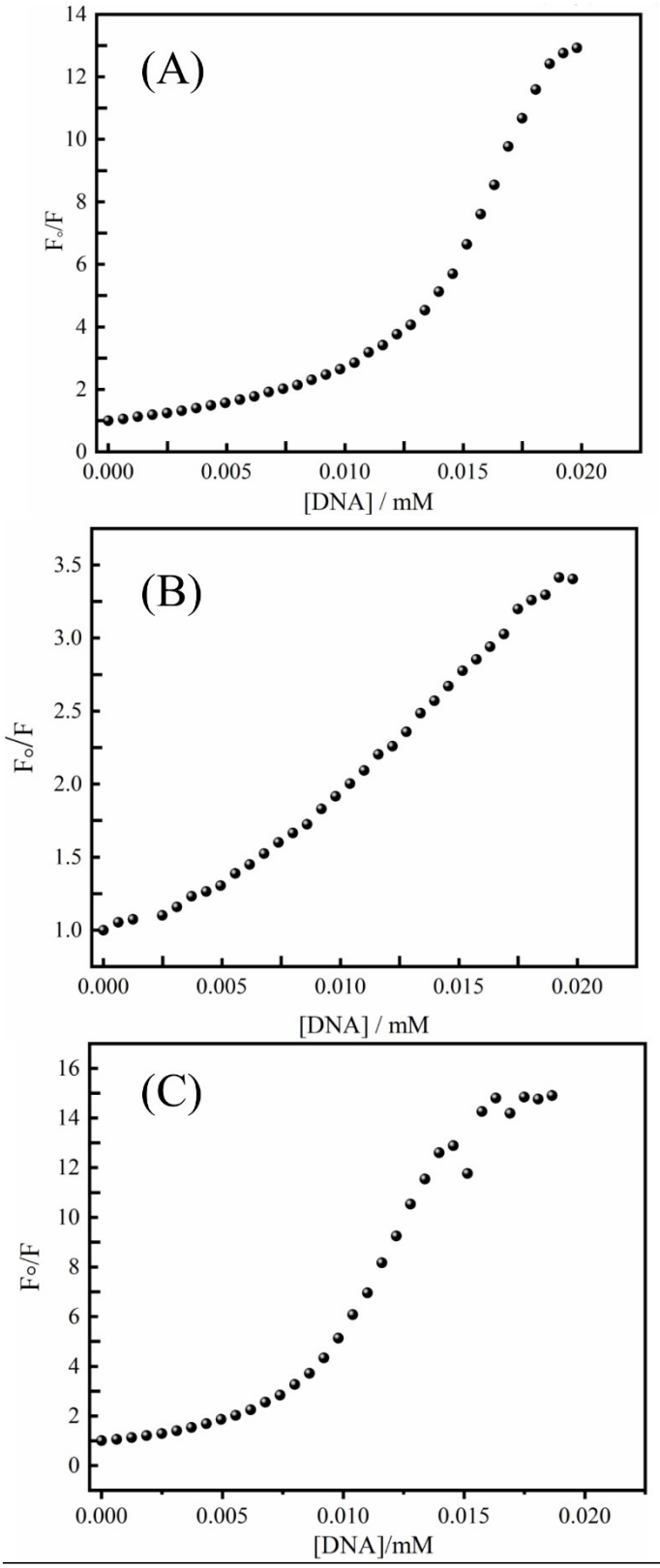
**Supporting Information for**

# Structural and thermodynamic insights into the Cren7 mediated DNA organization in Crenarchaeota

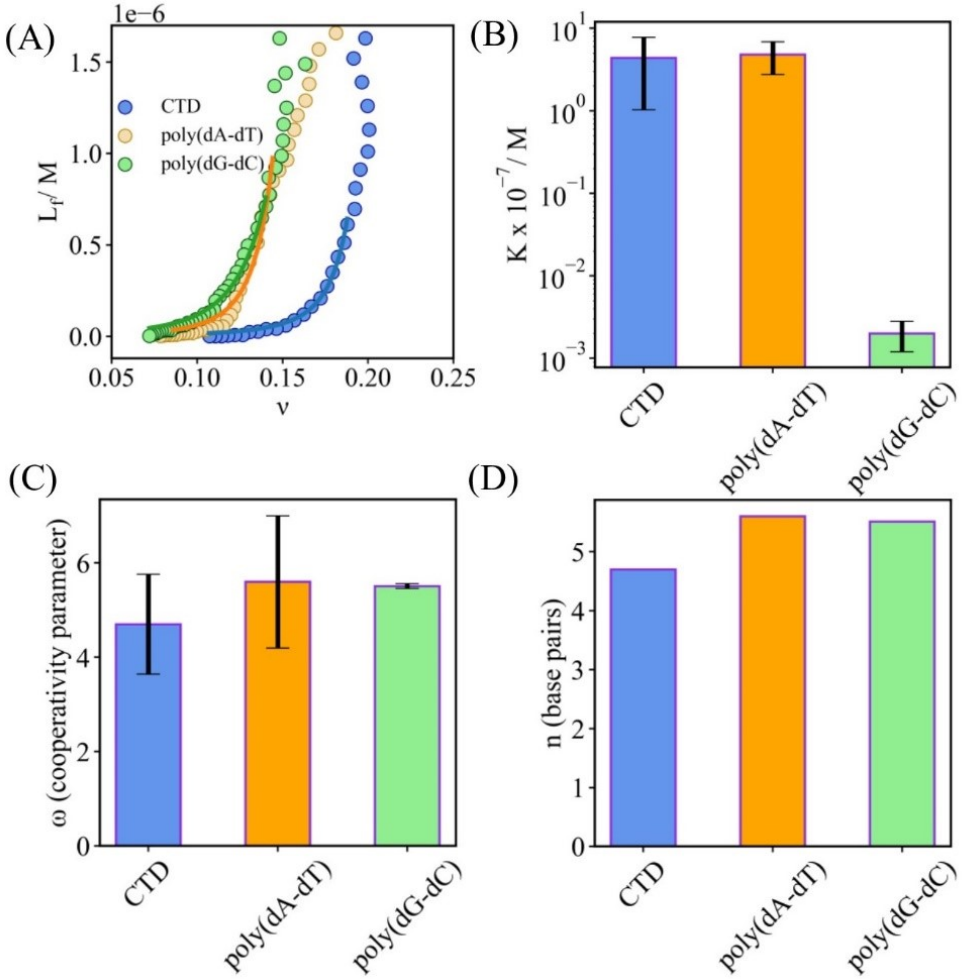
*Geetika K., Angel Rose Thomas, T. Srividya Vyjayanthi, Soumit S. Mandal*.*

Department of Chemistry, Indian Institute of Science Education and Research (IISER), Tirupati 517507, India.

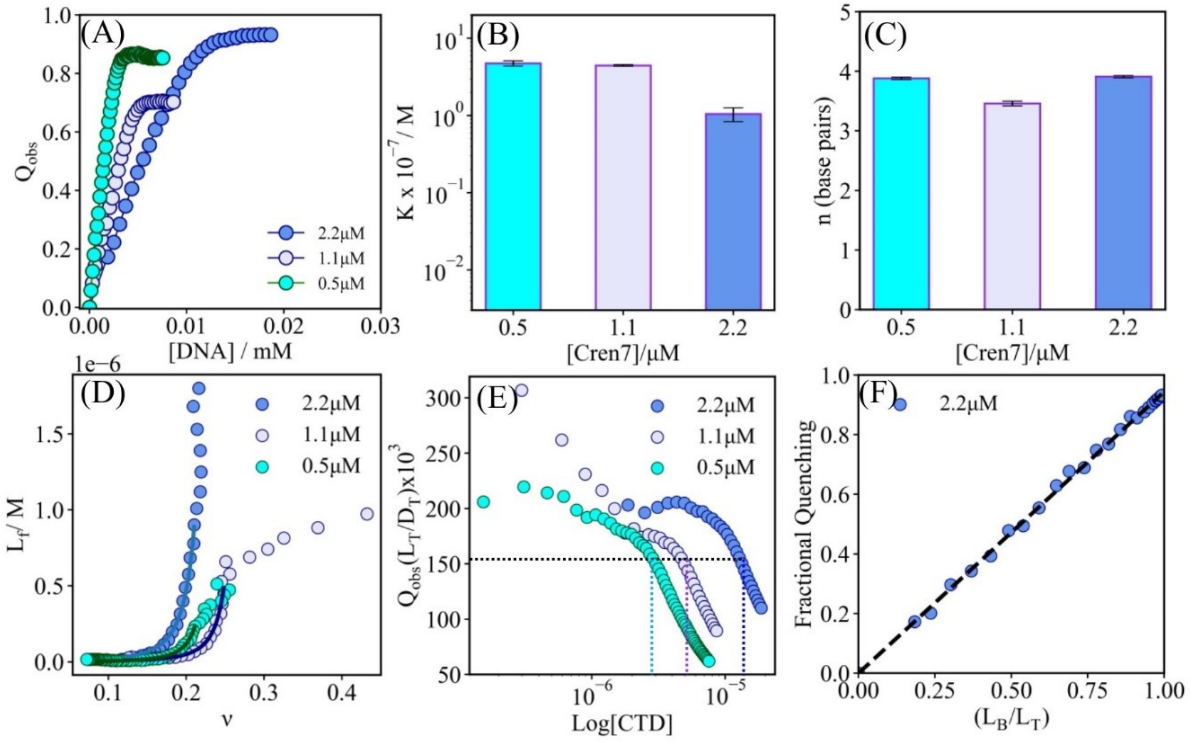
*To whom correspondence should be addressed: Soumit S. Mandal (Email: soumit.mandal@iiserirupati.ac.in)



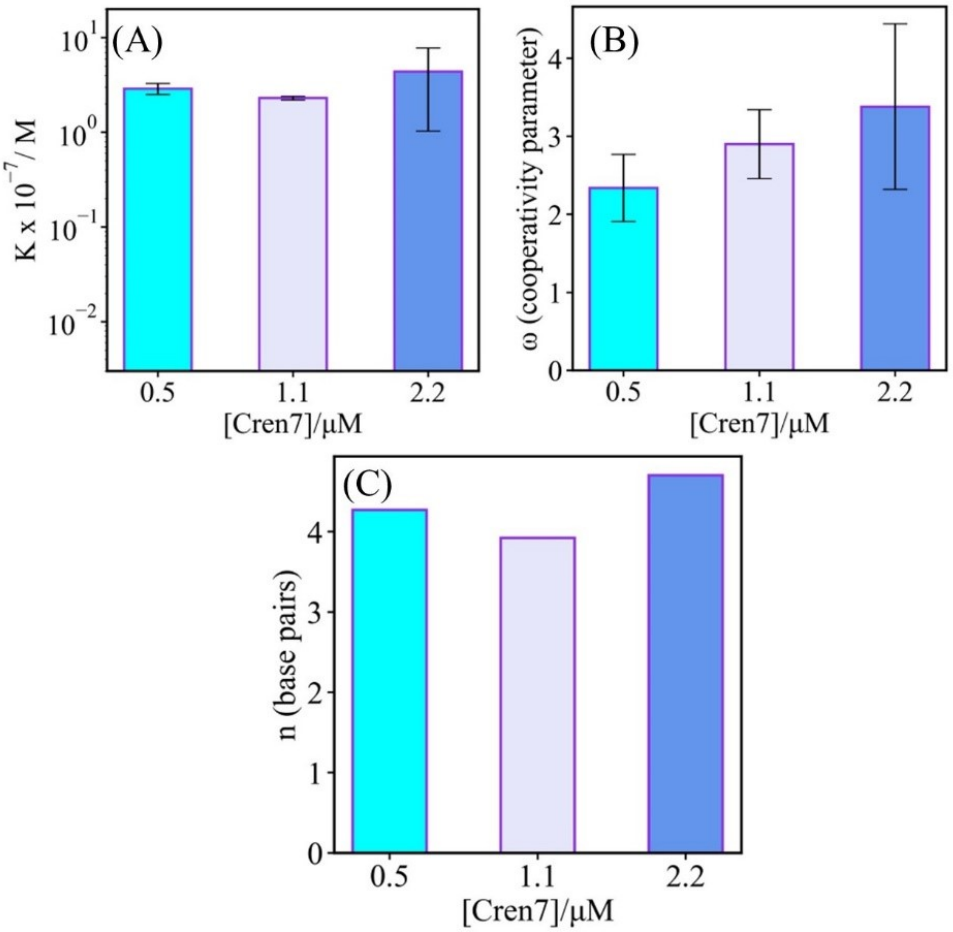

**Supplementary Figure 1**: Stern-Volmer plots representing the quenching of the Cren7 fluorescence during the reverse titration by (A) poly(dA-dT)·poly(dA-dT) (B) poly(dG-dC)·poly(dG-dC) and (C) CTD



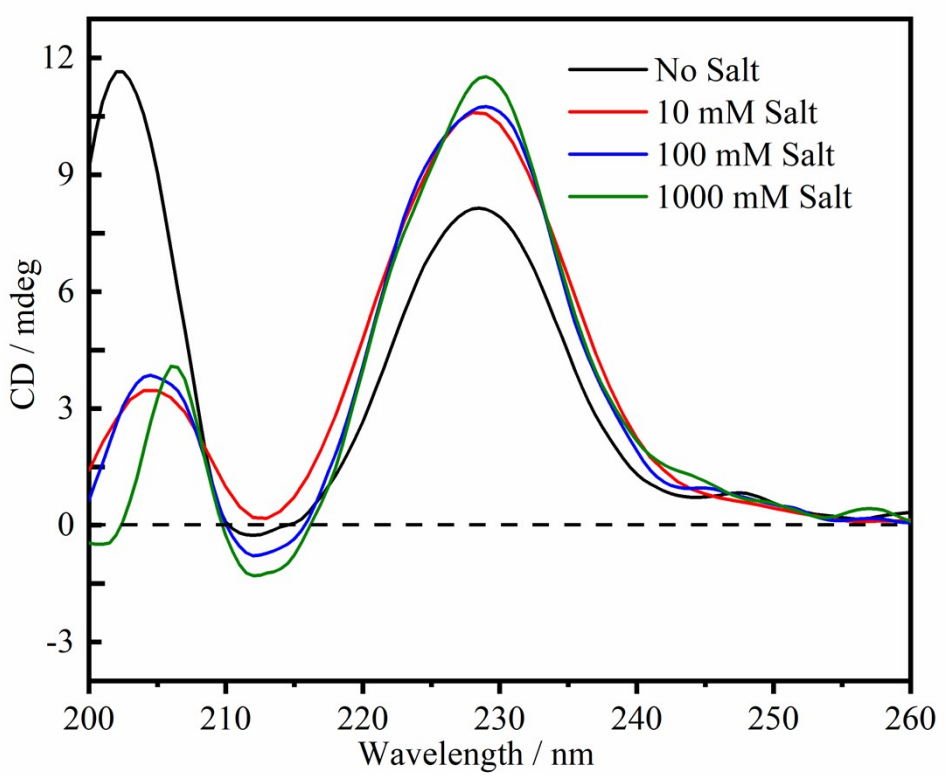

**Supplementary Figure 2:** (A) Plot describing the variation in the concentration of free, unbound Cren7, $L_f$ with the binding density function (moles of bound Cren7 per mole of total DNA lattice residues) ν during the reverse titration. CTD, poly(dA-dT)·poly(dA-dT) and poly(dG-dC)·poly(dG-dC) were the DNA and polynucleotides used in the reverse titration, respectively. The continuous line represents the least square fitting analysis performed with cooperative McGhee and von Hippel model. The parameters obtained from the fitting analysis are plotted in B-D for comparison. The binding affinity is plotted in (B), the cooperativity parameter in (C), and the binding site size in (D). The analysis was carried out assuming the binding site size obtained using ω=1 and it was kept invariant during this analysis.



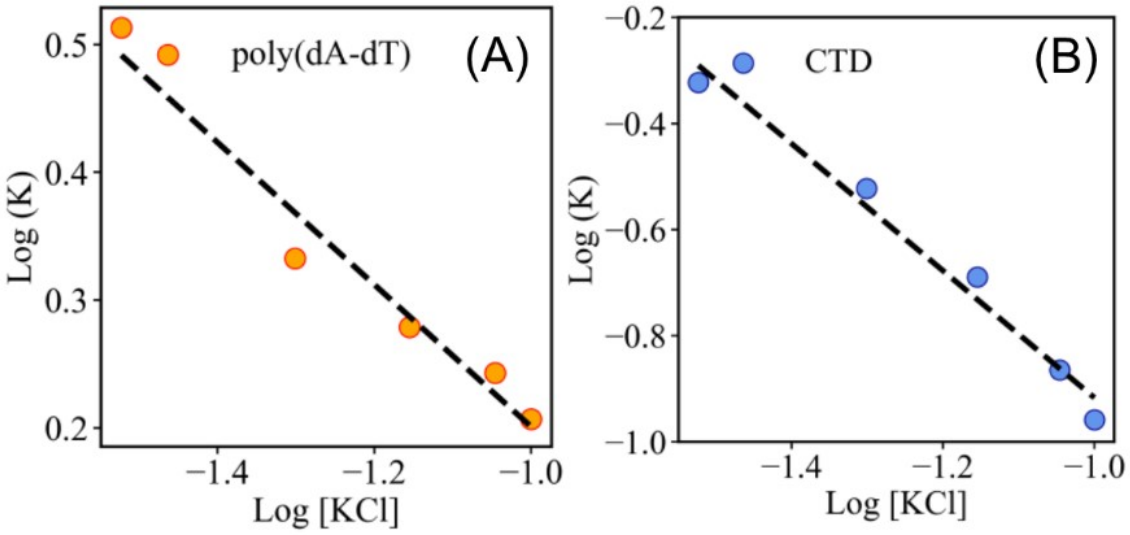

**Supplementary Figure 3: (**A) Observed fluorescence quenching ($Q_{obs}$) of Cren7 that arises due to its titration with increasing concentration of Calf Thymus DNA (CTD). The Cren7 concentration that was titrated with DNA varied between 0.55 to 2 μM. The titration profiles were analysed using a non-cooperative McGhee and von Hippel model to obtain the binding affinity and binding site size, which are plotted in (B) and (C), respectively. (D) Plot describing the variation of free unbound Cren7, $L_f$ with the binding density function (moles of bound Cren7 per mole of total DNA lattice residues) ν in the titration. The continuous line is the theoretical plot using the parameters obtained with the non-cooperative McGhee and von Hippel model. (E) The variation in the binding density parameter obtained for the different concentrations of Cren7 used in (A) is plotted as a function of the CTD concentration. The horizontal line (black dotted) at a certain value of $Q_{obs}(L_t/D_t)$ is the constant binding density function that would be obtained for different ratios of Cren7 and CTD concentration. The CTD concentrations corresponding to each Cren7 concentration are obtained from the point of intersection of cyan dotted (0.5μM), indigo dotted (1.1μM) and blue dotted for (2.2μM) lines on the x-axis. (F) The degree of fluorescence quenching ($Q_{obs}$) plotted against fraction of the bound Cren7 ($L_B/L_T$).



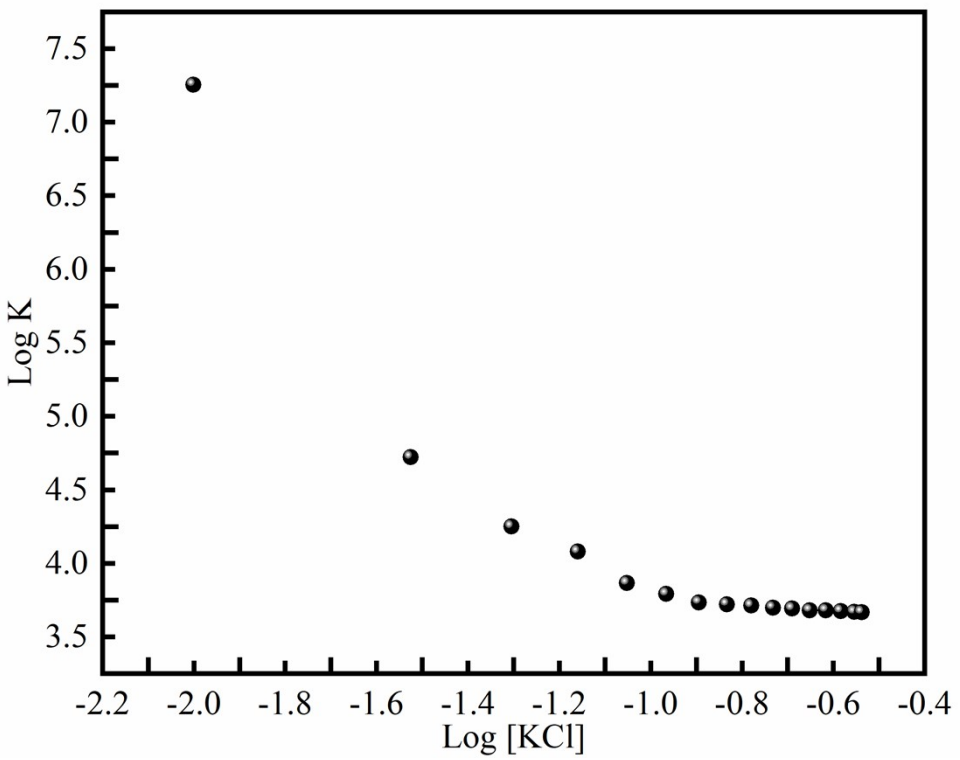

**Supplementary Figure 4:** Reverse titration profiles for different concentrations of Cren7 with the CTD were analysed with cooperative McGhee and von Hippel model. The parameters obtained from the analysis were plotted here. Plot describing the variation in the affinity constants (A) cooperativity parameter, ω (B) and binding site size, n (C) with different concentration of Cren7. The titration profiles were analysed with a cooperative McGhee and von Hippel model.



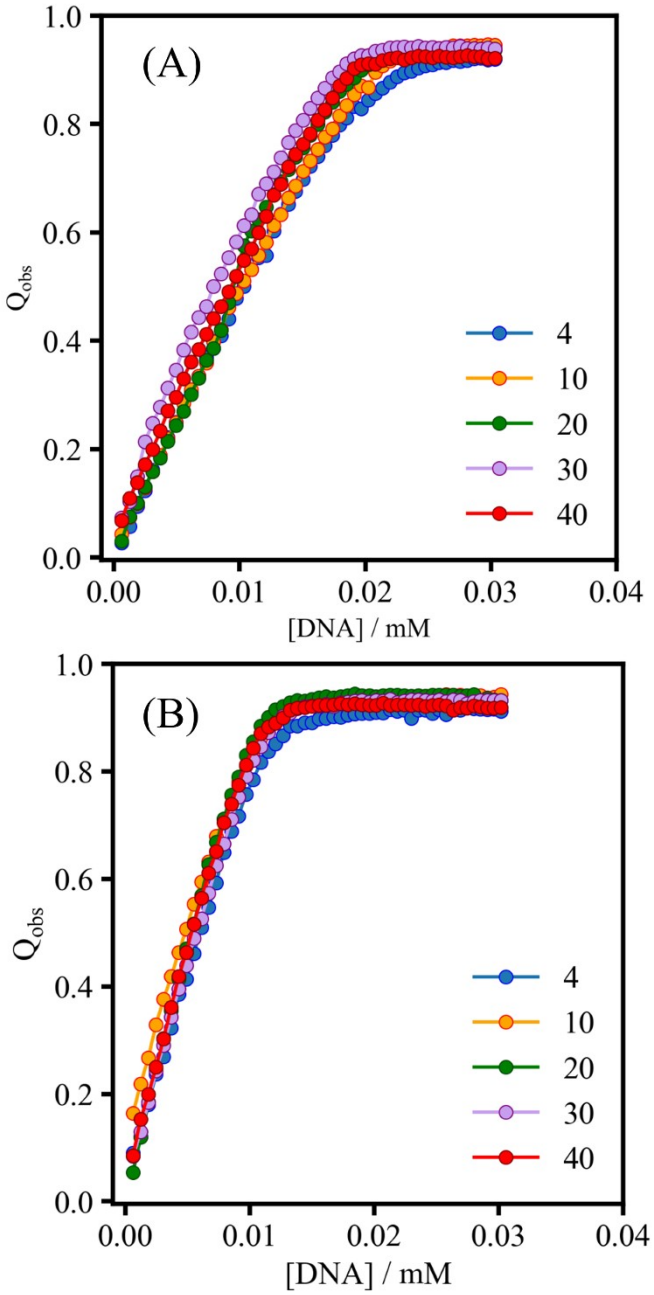

**Supplementary Figure 5:** Secondary CD of the Cren7 at different salt concentrations




**Supplementary Figure 6:** The reverse titration of Cren7 was performed with poly(dA-dT) and CTD in a buffer solution with KCl whose concentration was varied between 10-100 mM. The titration profiles were analysed using a noncooperative Mcghee von Hippel model to obtain the binding affinity which was plotted as a function of KCl concentration.




**Supplementary Figure 7**: Salt dependent binding affinity of Cren7 to poly(dGdC)·poly(dGdC) determined by salt back-titration.




**Supplementary Figure 8:** Reverse titration of Cren7 was performed in the fluorescence spectrometer with (A) polynucleotide, poly(dA-dT)·poly(dA-dT) and (B) CTD respectively at temperatures in the range 4-40 °C in 20 mM Sod.Phosphate buffer (pH 7.0).

**Supplementary Table 1:** Binding parameters of Cren7 resulting from their interaction with various nucleic acids[a]

| DNA | $K \times 10^{-7}$ / $M^{-1}$ | Site Size / bp | $Q_{max}$ |
|---|---|---|---|
| *Calf Thymus DNA* | 1.04 (±0.21)[b] | 2.05 (±0.06) | 0.94 |
| Poly(dAdT)·Poly(dAdT) | 0.37 (±0.06) | 2.37 (±0.06) | 0.93 |
| Poly(dGdC)·Poly(dGdC) | 0.12 (±0.08) | 2.21 (±0.05) | 0.76 |

[a] The binding parameters were estimated by reverse titrations using fluorescence quenching at 25 °C in 10 mM $KH_2PO_4$ (pH 7.4) without adding any other salt. The parameters were obtained by nonlinear least-squares fitting of fluorescence titrations described in the materials and methods section.

[b] Estimated errors are based on repeated measurements and represent the precision of the parameters from the nonlinear regression.

**Supplementary Table 2:** Variation in binding parameters of Cren7 to Poly(dA-dT). Poly(dA-dT) and Calf Thymus DNA due to the presence of different concentration of KCl.

| DNA type | Salt concentrations [a] | $K \times 10^{-6} / M^{-1}$ | Site Size / bp | $Q_{max}$ |
|---|---|---|---|---|
| Poly(dA-dT)· Poly(dA-dT) | 10 mM KCl | 6.39 (±0.59)[b] | 2.65 (±0.07) | 0.92 |
| | 100 mM KCl | 1.61 (±0.75) | 2.63 (±0.20) | 0.79 |
| | 1000 mM KCl | 0.249 (±0.02) | 3.54 (±0.18) | 0.55 |
| *Calf Thymus DNA* | 10 mM KCl | 1.18 (±2.69) | 1.99 (±0.07) | 0.93 |
| | 100 mM KCl | 0.11 (±0.001) | 2.17 (±0.11) | 0.89 |
| | 1000 mM KCl | 0.08(±0.001) | 3.09(±0.23) | 0.36 |

**Supplementary Table 3:** Binding parameters of Cren7 resulting from their interaction with CTD. The concentration of Cren7 varies between 0.55-2.2μM

| [Cren7] | $K \times 10^{-7}$ / $M^{-1}$ | Site Size / bp | $Q_{max}$ |
|---|---|---|---|
| 0.55μM | 4.75(±0.38) | 3.88 (±0.02) | 0.85 |
| 1.1μM | 4.45 (±0.11) | 3.46 (±0.04) | 0.70 |
| 2.2μM | 1.04 (±0.21) | 3.91 (±0.02) | 0.94 |

**Supplementary Table 4:** The temperature-dependent variation of binding parameters arises due to the Cren7 binding to Poly(dA-dT). Poly(dA-dT)a

| Temp / °C | K × $10^{-7}$ / $M^{-1}$ | Site Size / bp | $Q_{max}$ |
|---|---|---|---|
| 4 | 0.83 (±0.011)[b] | 3.48 (±0.09) | 0.93 |
| 10 | 2.34 (±1.73) | 3.73 (±0.08) | 0.95 |
| 20 | 4.32 (±2.17) | 3.54 (±0.00) | 0.94 |
| 30 | 1.10 (±0.37) | 2.96 (±0.10) | 0.95 |
| 40 | 2.81 (±0.84) | 3.37 (±0.08) | 0.93 |

[a]The binding parameters were estimated by reverse titrations using fluorescence in 10 mM $KH_2PO_4$, pH 7.4.b The errors were estimated as described in the footnotes of table1.

**Supplementary Table 5:** Temperature dependent variation of binding parameters arising due to the Cren7 binding to Calf Thymus DNA

| Temp / °C | K × $10^{-7}$ / $M^{-1}$ | Site Size / bp | $Q_{max}$ |
|---|---|---|---|
| 4 | 1.28 (±0.41) | 2.51 (±0.07) | 0.92 |
| 10 | 0.62 (±0.48) | 1.72 (±0.09) | 0.95 |
| 20 | 1.26 (±2.8) | 1.88 (±0.07) | 0.95 |
| 30 | 3.04 (±0.18) | 2.13 (±0.04) | 0.94 |
| 40 | 0.96 (±0.34) | 1.85 (±0.04) | 0.94 |

**Supplementary Table 6**: The cooperativity parameter obtained from the analysis of forward titration data obtained using a CD spectroscopy using a Hills model. Due to the binding of Cren7, a cooperative structural transition occurs in the polynucleotides and DNA.

CTD

| Salt Concentration | Cooperativity |
|---|---|
| No Salt | 3.89±0.58 |
| 10mM | 2.91±0.60 |
| 100mM | 3.80±1.30 |

Poly(dA-dT)·Poly(dA-dT)

| Salt Concentration | Cooperativity |
|---|---|
| No Salt | 13.67±3.81 |
| 10mM | 8.49±2.58 |
| 100mM | 9.77±4.68 |

Poly(dG-dC)·Poly(dG-dC)

| Salt Concentration | Cooperativity |
|---|---|
| No Salt | 1.90±0.18 |
| 10mM | 1.90±0.14 |
| 100mM | 0.92±0.51 |

**Supplementary Table 7:** The variation in the binding affinity obtained from the analysis of

| DNA type | Salt concentrations [a] | $K \times 10^{-6} / M^{-1}$ |
|---|---|---|
| Poly(dA-dT) · Poly(dA-dT) | 10 mM KCl | 6.39 (±0.59) |
| | 30 mM KCl | 3.26(±1.29) |
| | 50 mM KCl | 2.15(±1.8) |
| | 70 mM KCl | 1.9(±0.3) |
| | 90 mM KCl | 1.75(±0. 9) |
| | 100 mM KCl | 1.61 (±0.75) |
| *Calf Thymus DNA* | 10 mM KCl | 1.18 (±2.69) |
| | 30 mM KCl | 0.47(±1.09) |
| | 50 mM KCl | 0.30(±0.6) |
| | 70 mM KCl | 0.20(±0.3) |
| | 90 mM KCl | 0.13(±0.16) |
| | 100 mM KCl | 0.11 (±0.001) |

the reverse titration of Cren7 with CTD at diff