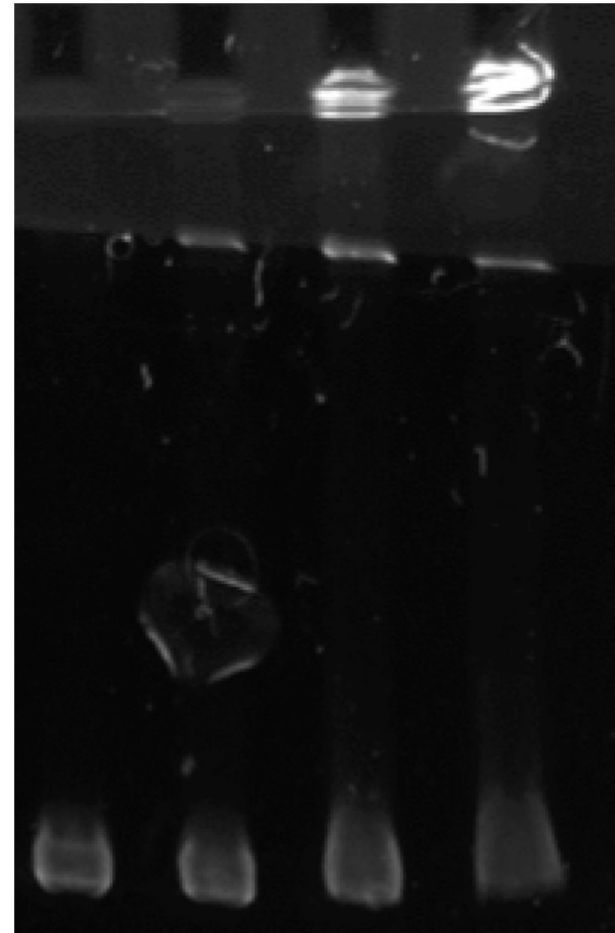


**Sup. figure S1:**  $K_D$  measurements of WT Hfq-CTR:dsDNA complexes using fluorescence anisotropy in the presence of 100 mM NaCl, 300 mM NaCl and 1 mM MgCl<sub>2</sub>. Hfq-CTR without salts added (black) has an equilibrium dissociation constant  $K_D = 260 \pm 10$  nM, while in the presence of 100 mM NaCl  $K_D = 240 \pm 11$  nM (blue). Increasing salt concentration to 300 mM increases  $K_D$  to  $455 \pm 20$  nM (red) and thus reduce the stability of the complex. MgCl<sub>2</sub> at 1 mM does not change significantly the affinity ( $K_D = 259 \pm 9$  nM, green)

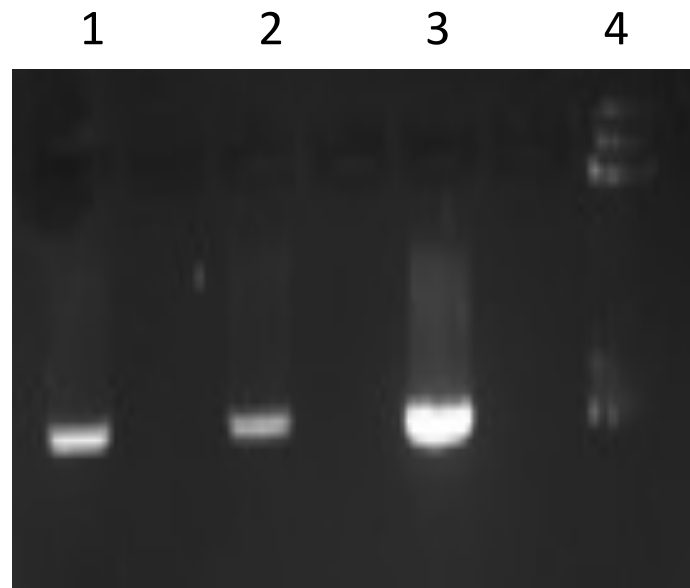


← complexes  
(CTR<sub>n</sub>:AT<sub>59</sub>)<sub>n</sub> ?

← complex  
(CTR<sub>n</sub>:AT<sub>59</sub>)?

← free DNA

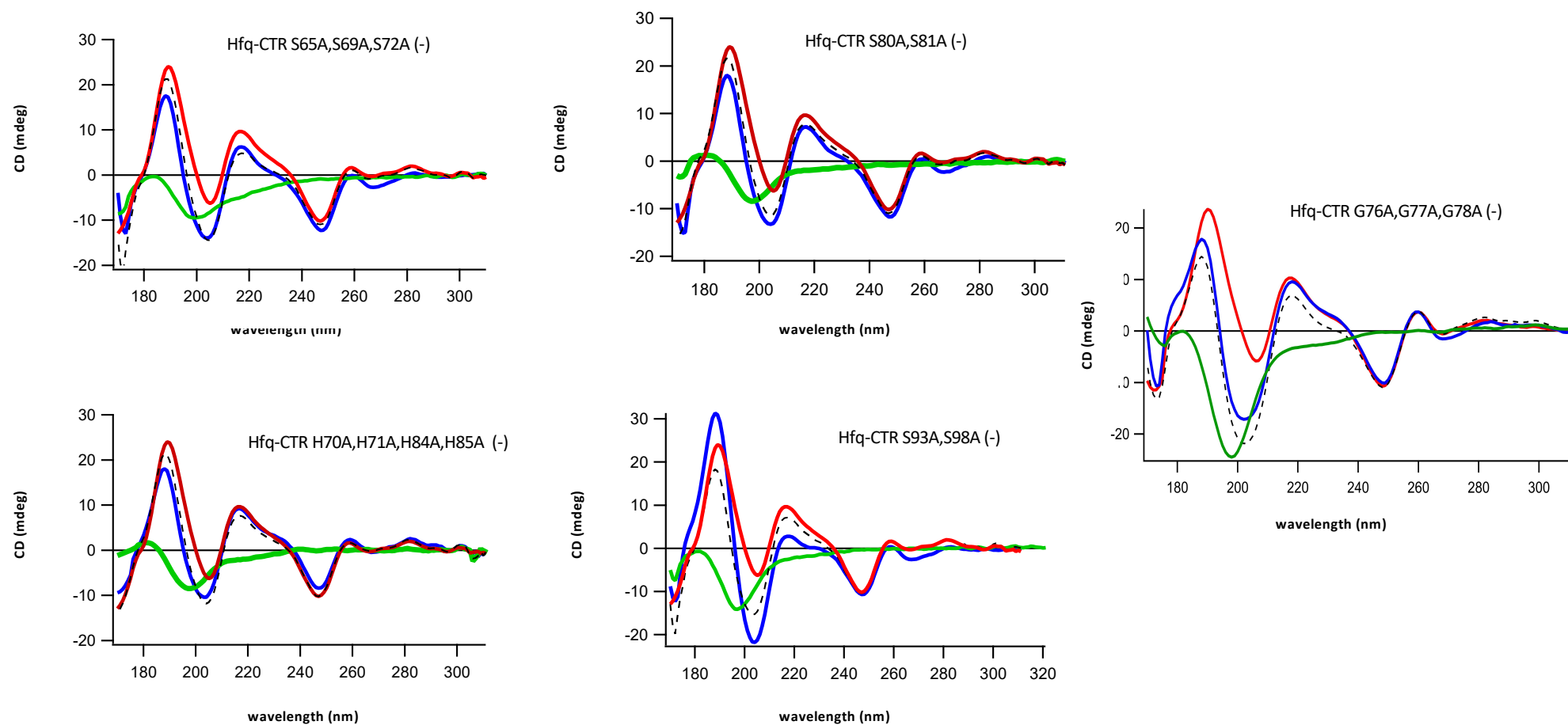
**Sup. figure S2:** EMSA analysis of (dA:dT)<sub>59</sub> titration by Hfq-CTR. Increasing CTR concentration to titrate DNA results in very high molecular weight complexes (possibly (CTR<sub>n</sub>:AT<sub>59</sub>)<sub>n</sub>) that stay in the well of the gel and cannot be quantified accurately.



**Sup. figure S3:** EMSA analysis of additional Hfq-CTR mutants in the presence of DNA. 1: DNA alone; 2: DNA + Hfq-CTR H70A,H71A; 3: DNA + Hfq-CTR H84A,H85A; 4: DNA + Hfq-CTR D97A,E99A,E100A,E102A. The increase in the band intensity in 3 may be due to the binding of few Hfq-CTR H84A,H85A monomers to DNA not detected by band-shift (Wang H. & Myong, S. Protein induced fluorescence enhancement PIFE for probing protein-nucleic acid interactions. *Chem Soc Rev* **2014**, 43, 1221-1229)



**Sup. figure S4:** EMSA analysis of pre-polymerized Hfq-CTR in the presence of DNA. 1: DNA alone; 2: DNA + pre-polymerized Hfq-CTR 100 nM; 3: DNA + pre-polymerized Hfq-CTR 1  $\mu$ M. Due to the aggregation of the protein, it was not possible in this case to measure  $K_d$  using fluorescence anisotropy.



**Sup. figure S5: SRCD spectra of additional CTR/DNA complexes.** Spectra of the individual components are measured with equivalent DNA and CTR concentrations. Complex: blue, DNA: red and CTR: green. The dotted spectrum represents the relevant combination of the spectra pertaining to the individual components. (-) indicate the behavior observed, in these cases no significant effect. Hfq-CTR S80A,S81A mutant spectra was reprinted with permission from *Biomacromolecules* 2020, 21, 3668-3677. Copyright 2020 American Chemical Society.