Atomic force microscopy

For AFM, a support substrate of freshly cleaved mica was functionalized with a 167 μM solution of 1-(3-aminopropyl)-silatrane, as described previously [75]. Cra and FruK protein samples were mixed with DNA at the indicated concentrations in 1x Cra Buffer (50 mM HEPES, 100 mM KCl, 0.1 mM EDTA, 10 mM $MgCl_2$, 0.3 mM DTT, pH 7.0) and incubated for 60 minutes at room temperature. After incubation, a 1:4 dilution of each sample was made using 1x Cra Buffer, of which 9 μL were immediately deposited on the functionalized mica surface. Deposited samples were incubated for 2 minutes, then rinsed with 200 μL ddH_2O and dried with a gentle stream of argon. A typical image of 1x1 μm^2 with 512 pixels/line was obtained under ambient conditions with a MultiMode AFM system (Bruker) using TESPA probes (Bruker Nano, Camarillo, CA, USA) housed at the Nanoimaging Core Facility at the University of Nebraska Medical Center. AFM images are shown in Supplemental Figure 5B, with the analyzed complexes indicated with boxes.

To analyze the particles observed in the AFM images, the volume of DNA-bound and DNA-free protein complexes were measured using AtomicJ [76]. The profile tool was used to obtain the following measurements: the complex height (h), the width at half-height of the long axis (w_{long}), and the width at half-height of the orthogonal axis (w_{orth}) (Supplemental Figure 5A). For DNA-bound complexes, the height (h_{DNA}) and width at half-height (w_{DNA}) were also obtained for DNA adjacent to each DNA-bound complex. To calculate the volume (V) from the AFM images, peaks corresponding to protein complexes were fit as an elliptic cylinder

$$V = \pi * h * r_{long} * r_{orth} \qquad \text{Equation 3}$$

with the radii (r_{long} and r_{orth}) being half of the measured widths. DNA was considered to be a cylindrical segment:

$$V = 2/3 * h_{DNA} * w_{DNA}^2 \quad \text{Equation 4}$$

For DNA-bound complexes, the volume of adjacent DNA was subtracted so that only the volume attributed to the protein(s) remained. (Two other geometric models were considered for the protein peaks, but neither could be matched to the expected molecular weights, below. (i) Treating the peaks as a segment of a sphere under-estimated the volumes compared to expected molecular weights. (ii) Treating the peaks as a rectangular box over-estimated the volumes compared to expected molecular weights.)

To convert volumes to molecular weight, the nm^3 volumes were divided by a conversion factor for the density. A value of 0.67 mL/g was determined from the crystal structures of both the Cra regulatory domain (PDB 2IKS;[22]) and ribokinase (PDB 1RKA; [77]; ribokinase is a homolog of FruK). The density values were obtained by dividing the solvent-excluded volume (calculated by the *surface* command within UCSF Chimera [78]) by the molecular mass of the atoms present (mass for missing residues was subtracted). On the plots, reference lines for higher order complexes were calculated from the theoretical molecular weights for monomeric Cra (37,999 Da) and monomeric FruK (33,756 Da).