Atomic Force Microscopy (AFM) Supplement.

Methodology

For AFM, a support substrate of freshly cleaved mica was functionalized with a 167 μM solution of 1-(3-aminopropyl)-silatrane, as described previously.[1] 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 below (Panel B Images I-VII), 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.[2] 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}) (Panel A). 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:

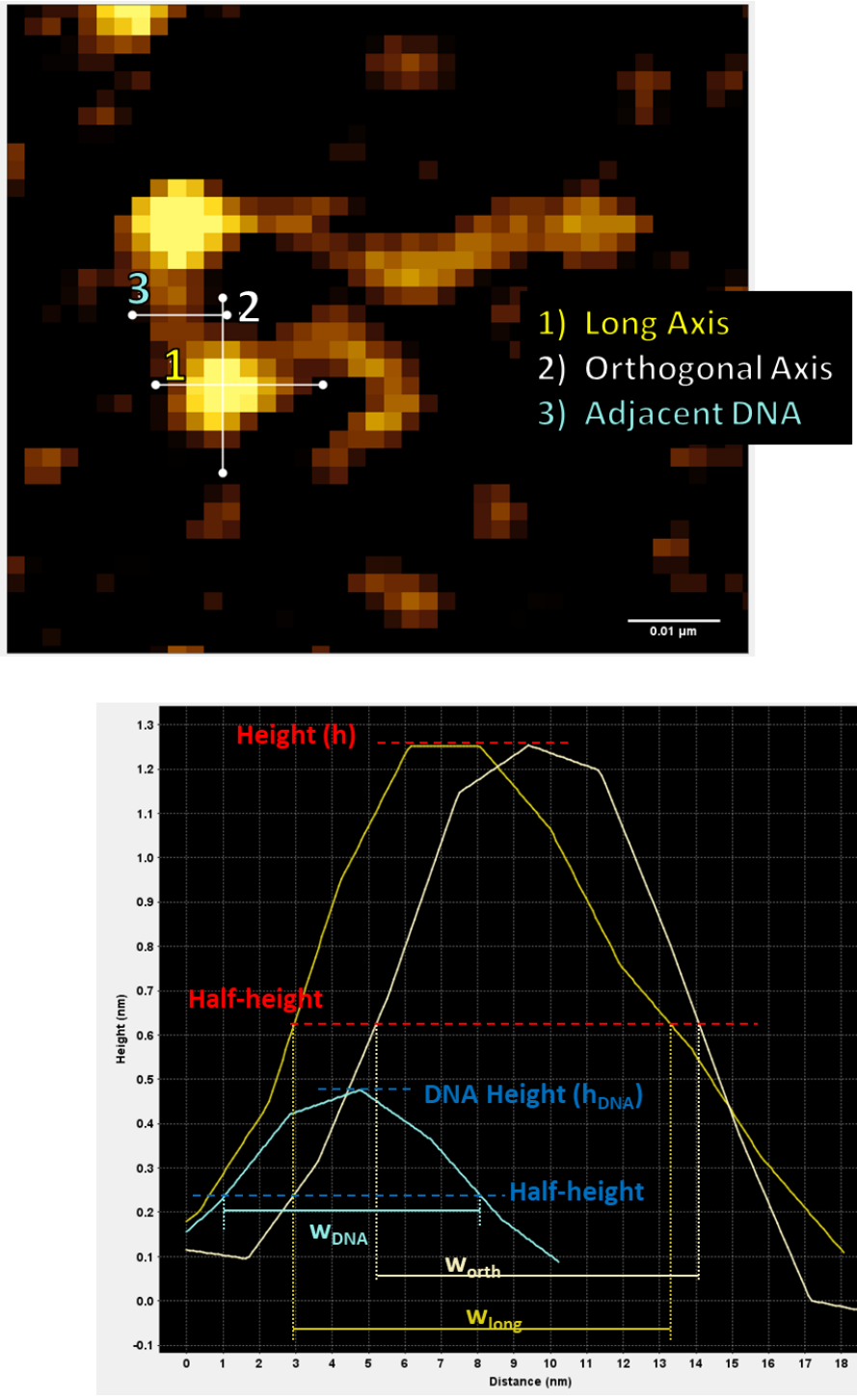
$$V = 2/3 * h_{DNA} * w_{DNA}^2 \qquad \text{Equation 4}$$

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;[3]) and ribokinase (PDB 1RKA[4]; 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[5]) 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).

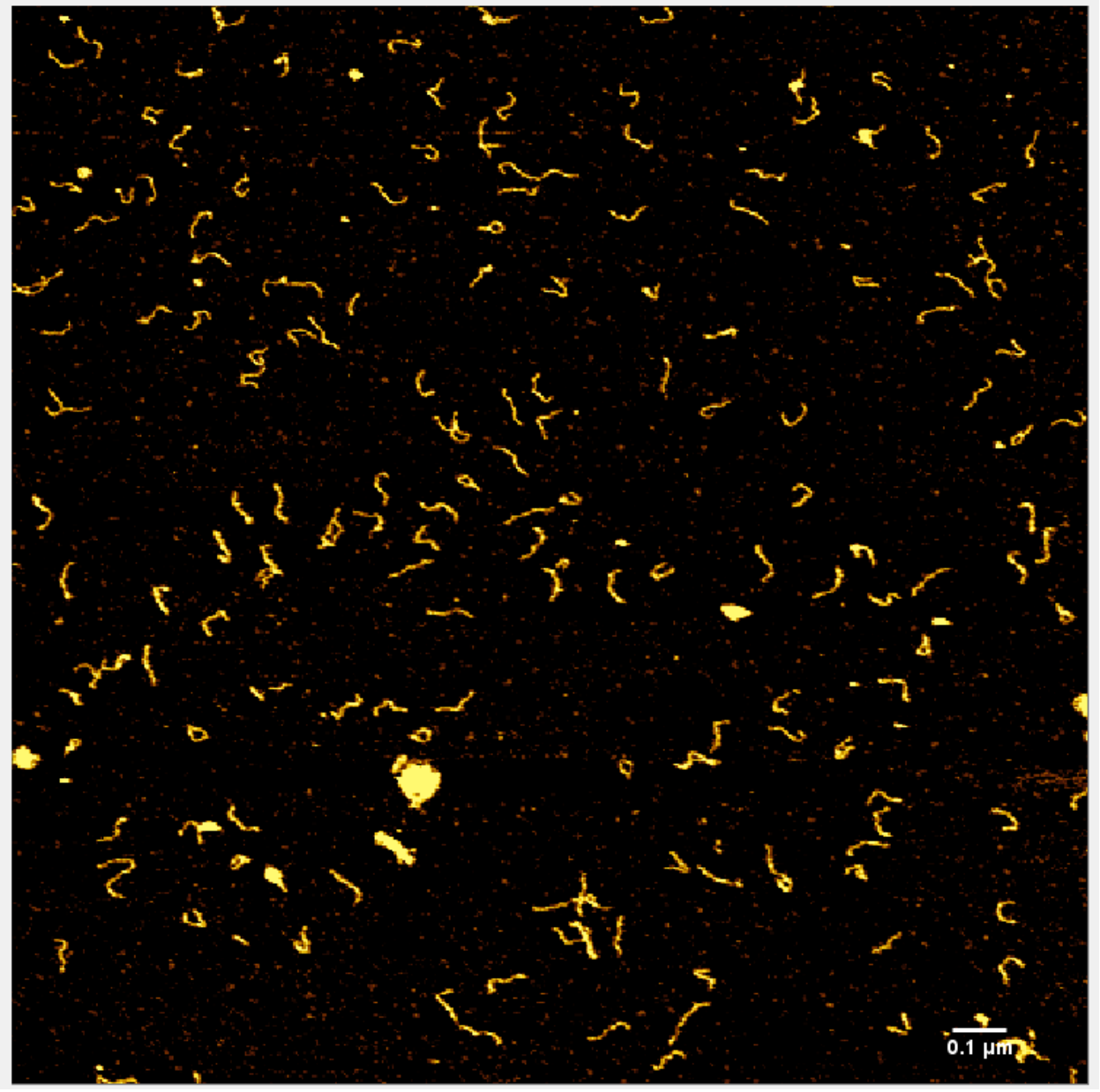
Figure

A. Parameters were measured on AFM images using the software AtomicJ [2] as illustrated below. The colors of the numbers in the top panel correspond to the peak colors in the bottom panel. Using these parameters, peak volumes were calculated using Equations 3 and 4 (main text).

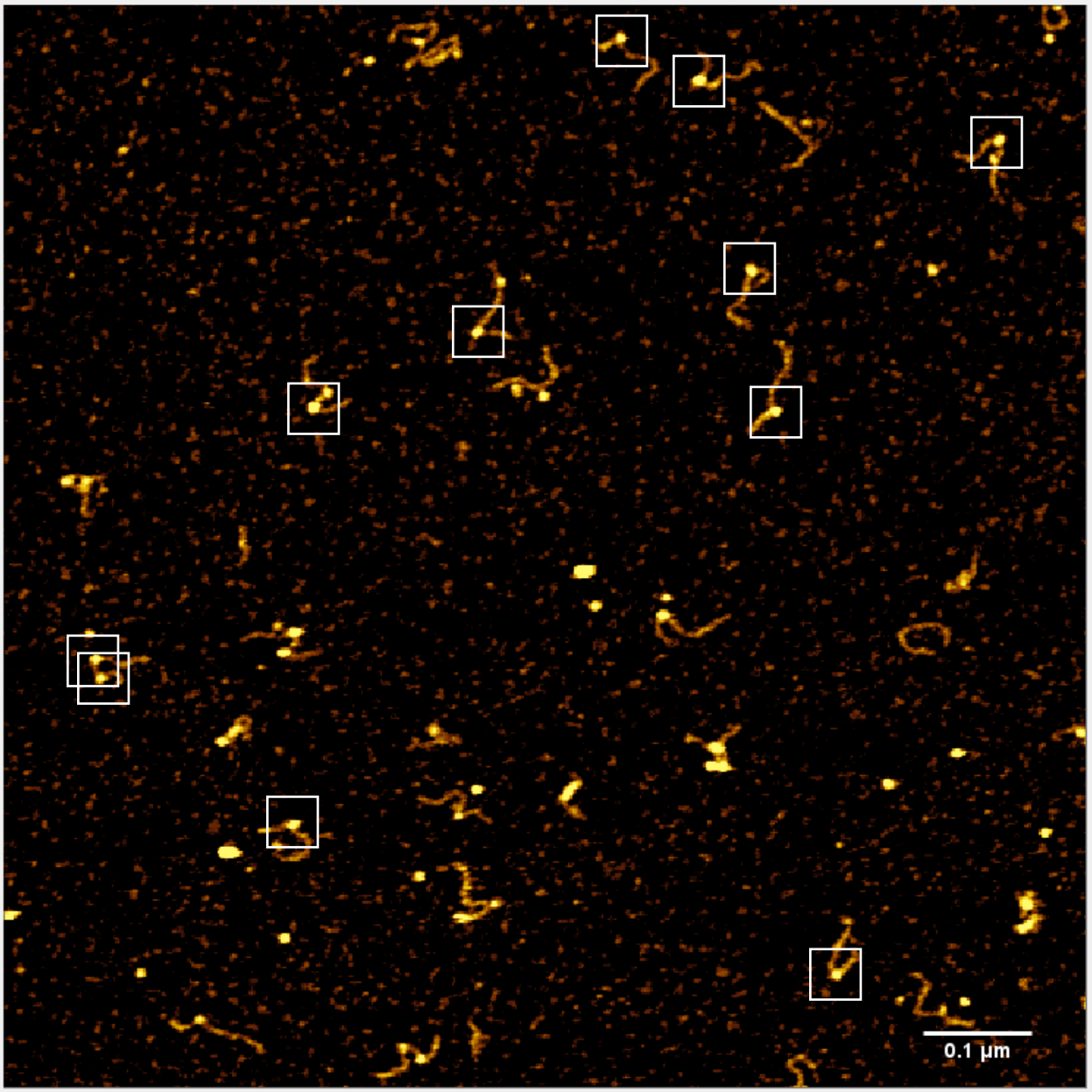


B. Full AFM images for each sample are shown on the following pages. Each image is displayed using the same depth scale from 0.2 to 1 nm; the actual maximum depth for most AFM images exceeded 3 nm. These widefield views show a 1x1 μm^2 AFM image unless otherwise indicated. Boxes (50x50 nm^2 unless otherwise indicated) indicate the complexes that were analyzed. Cra and FruK concentrations are provided as M dimer.

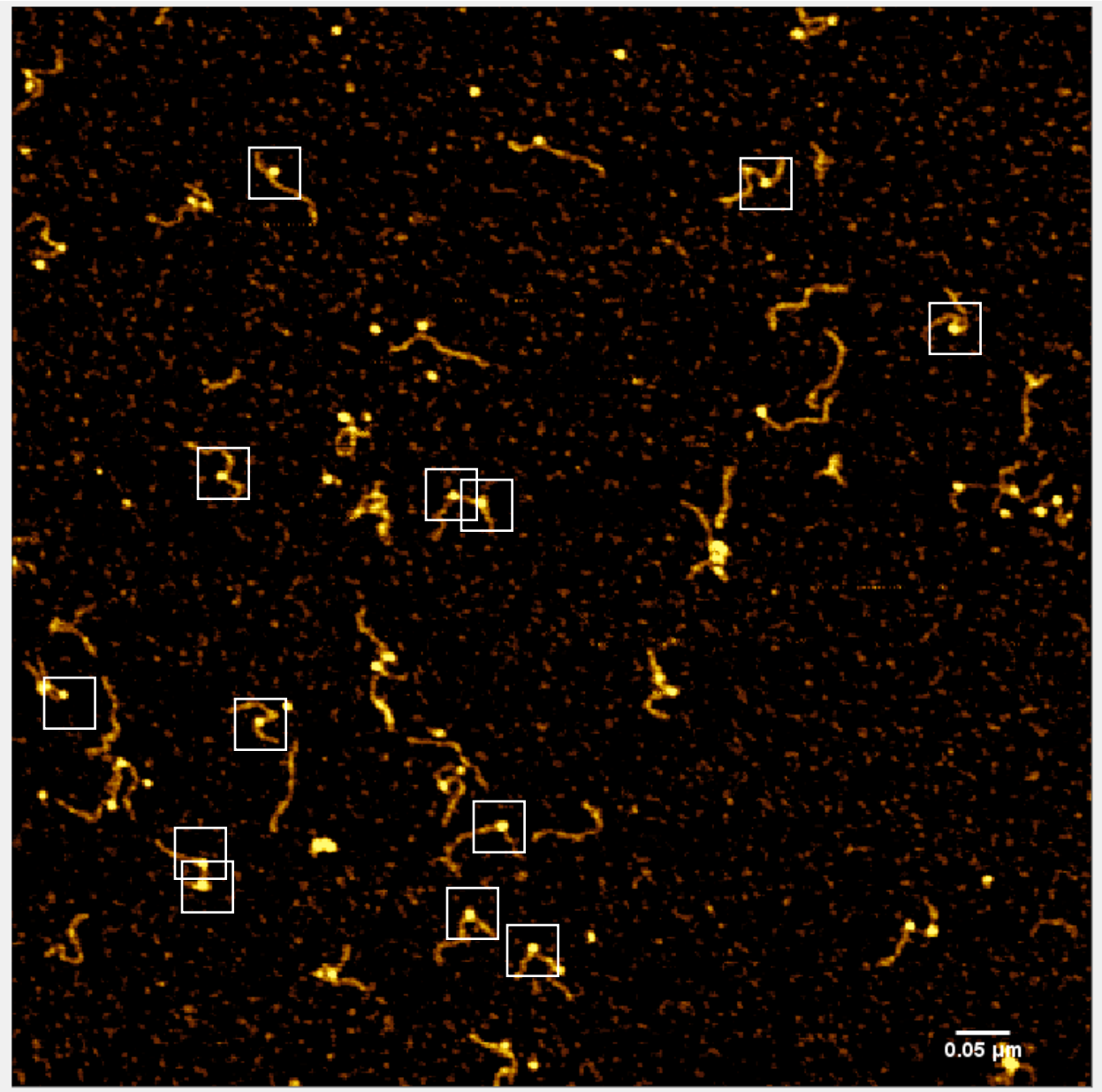
I. A sample of 10 nM DNA was diluted 1:4 (2x2 μm^2 AFM image) prior to adherence on functionalized mica. The DNA fragment (331 bp) was amplified from the cra binding region of the *fruBKA* operon.



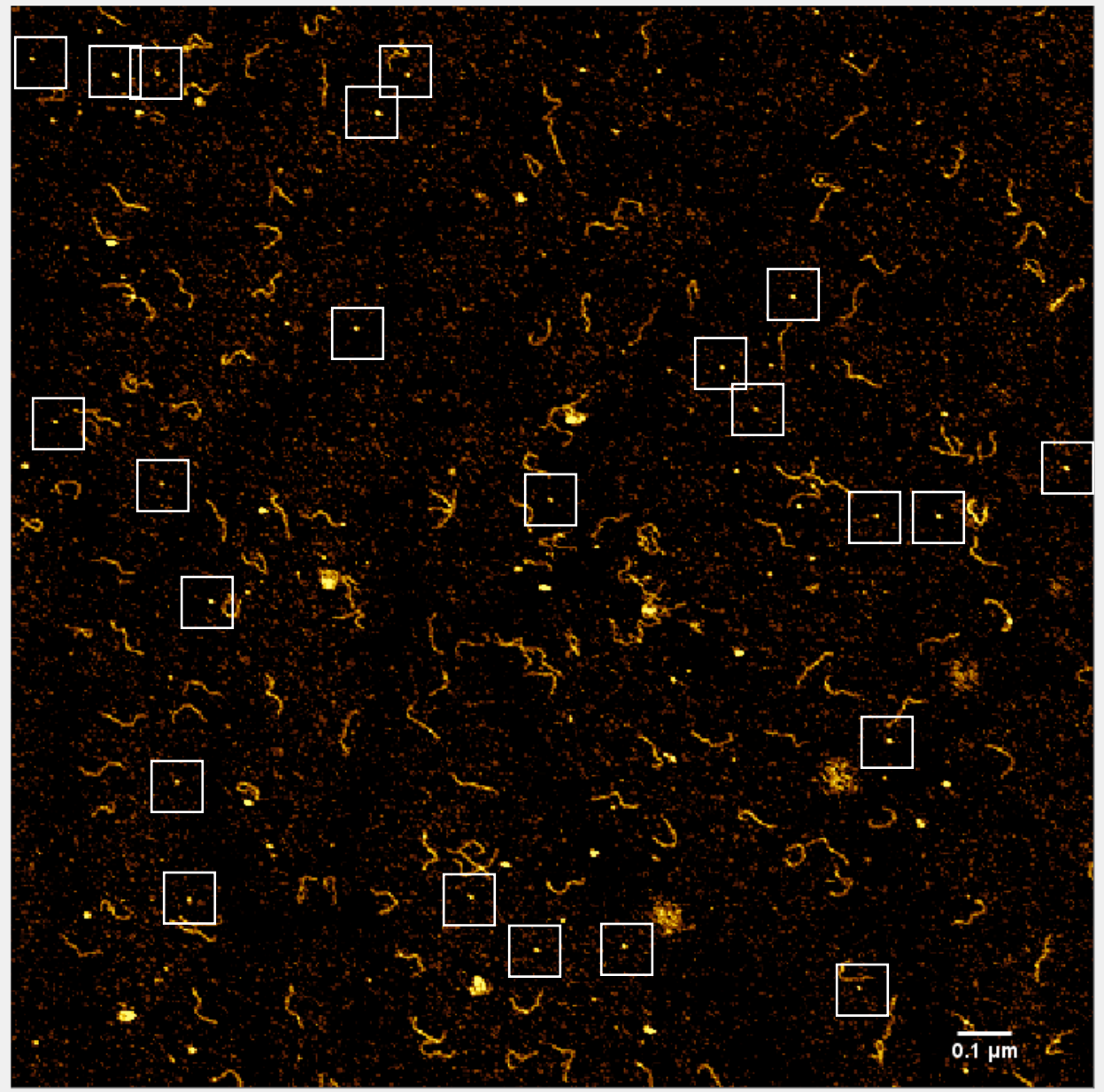
II. (S1-4a.001) A sample comprising 20 nM Cra and 10 nM DNA was diluted 1:4 prior to adherence on functionalized mica. Note the additional density on the DNA that was not observed for the DNA-only sample. Each 50x50 nm^2 box indicates an analyzed protein-DNA complex.



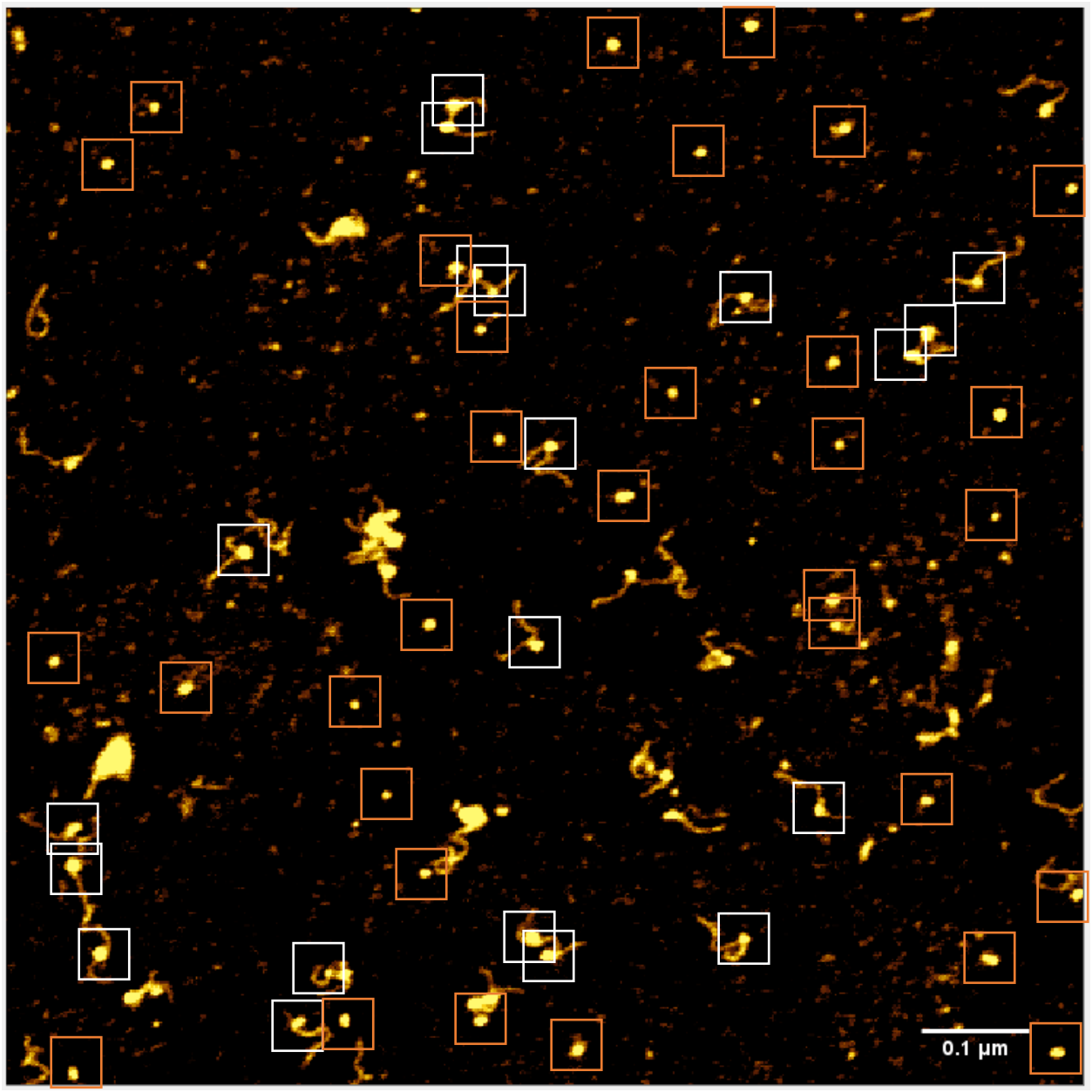
III. (S1-4a.002) Additional scan site of the 20 nM Cra/10 nM DNA sample.



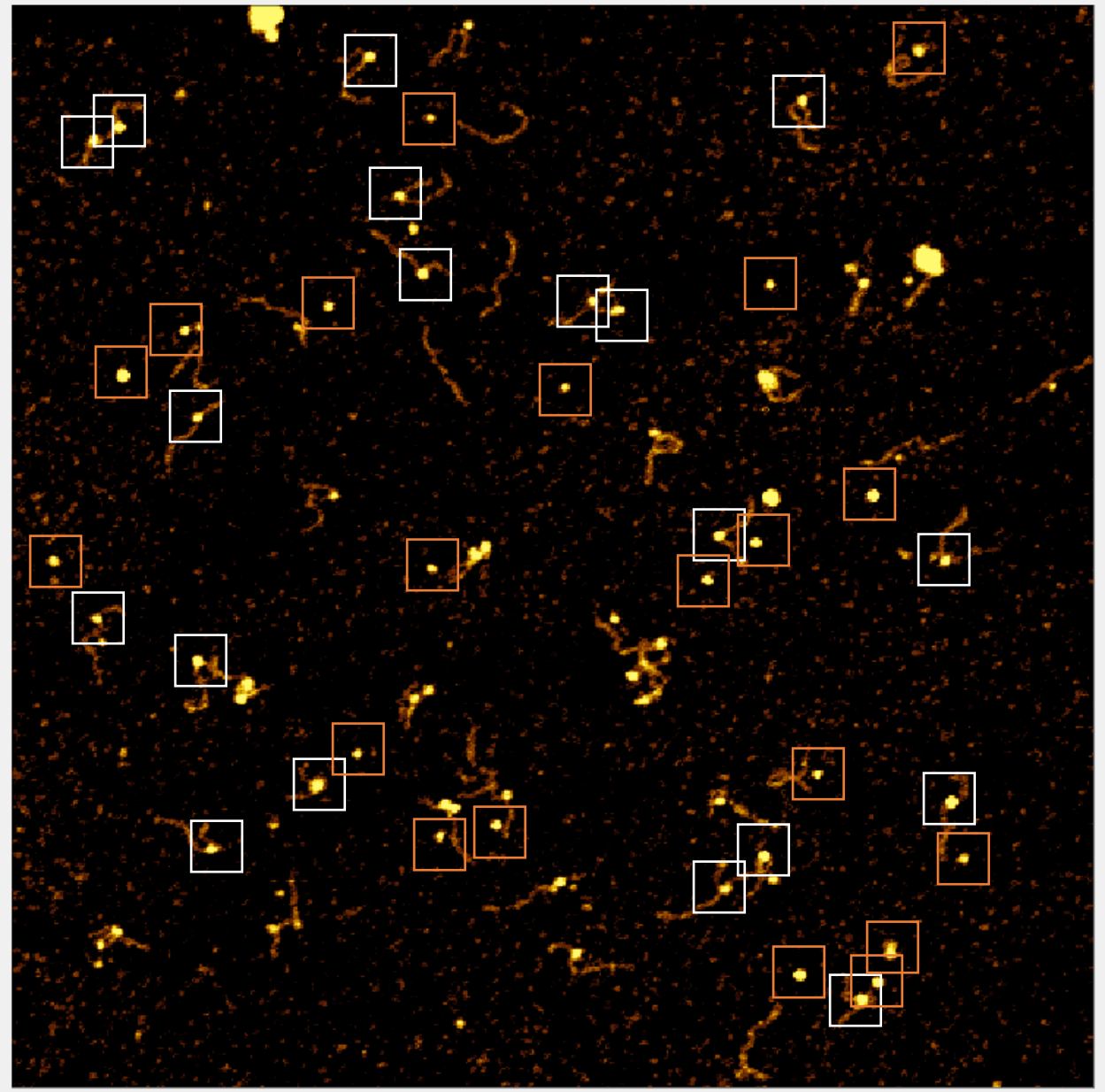
IV. (S2-4a.000) A sample comprising 20 nM FruK and10nM DNA was diluted 1:4 prior to adherence on functionalized mixa (2x2 μm^2 AFM image). Most DNA appears as in the DNA-only sample, with no significant FruK binding. Instead, multiple densities were observed in the background, which likely corresponds to free FruK. This behavior is consistent with other observations that FruK lacks the ability to bind to DNA. Each box (100x100 nm^2) indicates an analyzed, DNA-free protein complex.



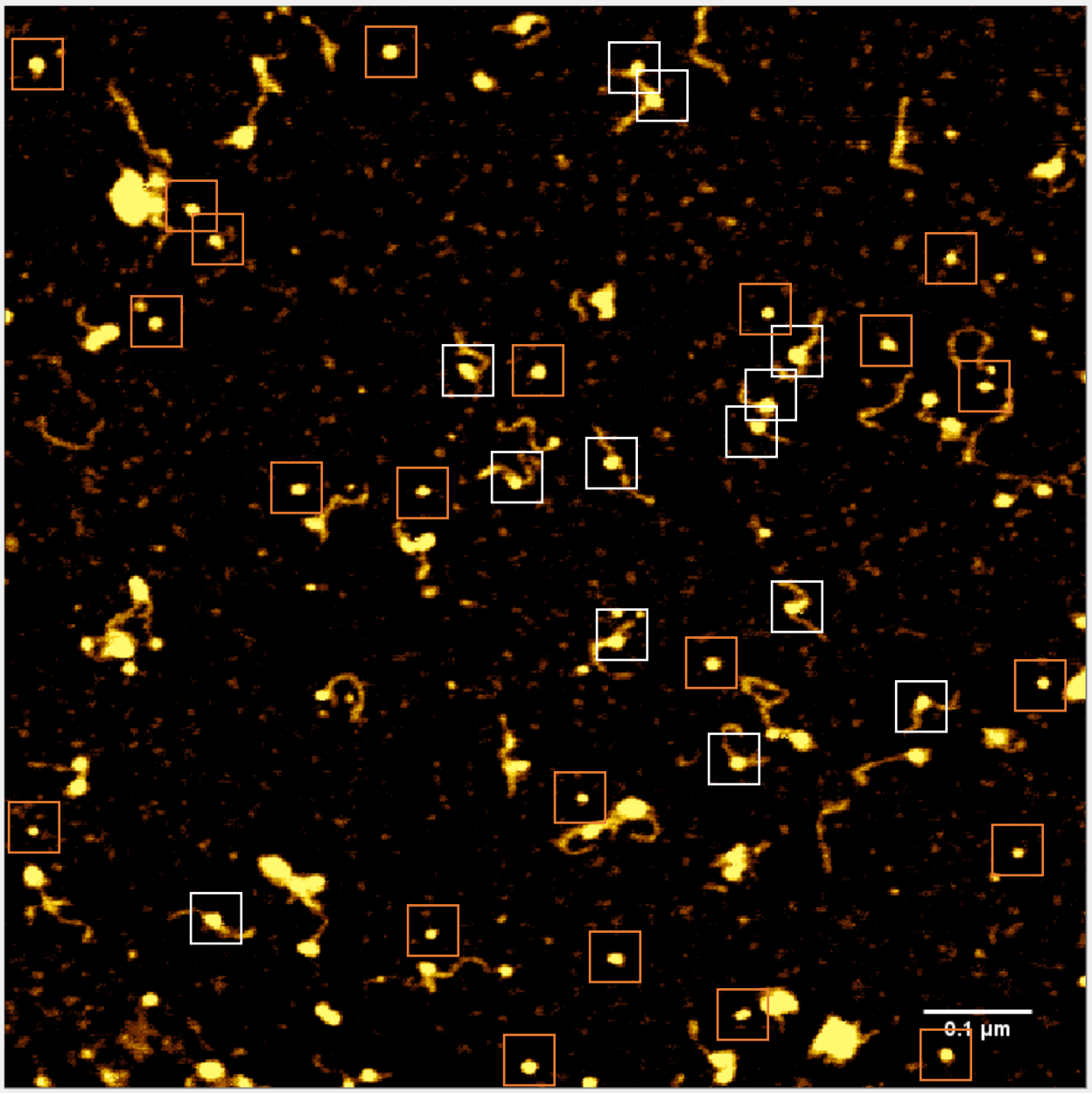
V. (S3-4a.001) A sample comprising 20 nM Cra, 20 nM FruK, and 10 nM DNA was diluted 1:4 prior to adherence on functionalized mica. As in the Cra/DNA sample, additional density was observed on the DNA (white boxes). When compared to the Cra/DNA sample, the DNA-bound density often appears larger, suggesting binding of FruK. In addition to density on the DNA, many complexes were observed in the background (orange boxes). When compared to either the Cra/DNA or FruK/DNA samples, this sample showed both larger and a greater number of these complexes, which suggests that their formation requires the presence of both Cra and FruK.



VI. (S3-4a.002) Additional scan site of the sample comprising 20 nM Cra, 20 nM FruK, and 10 nM DNA.



VII. (S4-4a.001) A sample comprising 20 nM Cra, 10 nM FruK, and 10 nM DNA was diluted 1:4 prior to adherence on functionalized mica. As in the equimolar FruK/Cra/DNA sample additional density was observed on the DNA. Strikingly, when compared to the samples shown in IV-V, the DNA-bound and DNA-free densities often appear larger. This sample with the lower FruK concentration also showed a greater number of large aggregates and/or higher order structures. White boxes indicate an analyzed DNA-bound protein complex; orange boxes indicate an analyzed DNA-free protein complex.



References cited

1 Lyubchenko, Y. L. & Shlyakhtenko, L. S. AFM for analysis of structure and dynamics of DNA and protein–DNA complexes. *Methods* **47**, 206-213 (2009). https://doi.org:https://doi.org/10.1016/j.ymeth.2008.09.002

2 Hermanowicz, P., Sarna, M., Burda, K. & Gabryś, H. AtomicJ: an open source software for analysis of force curves. *Rev Sci Instrum* **85**, 063703 (2014). https://doi.org:10.1063/1.4881683

3 Chang, C., Evdokimova, E., Kagan, O., Savchenko, A., Edwards, A.M., Joachimiak, A., Midwest Center for Structural Genomics (MCSG). Crystal structure of N-terminal truncated DNA-binding transcriptional dual regulator from Escherichia coli K12. *PDB DOI: 10.2210/pdb2IKS/pdb* (2006).

4 Sigrell, J. A., Cameron, A. D. & Mowbray, S. L. Induced fit on sugar binding activates ribokinase. *J Mol Biol* **290**, 1009-1018 (1999). https://doi.org:10.1006/jmbi.1999.2938

5 Pettersen, E. F. *et al.* UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **25**, 1605-1612 (2004). https://doi.org:10.1002/jcc.20084