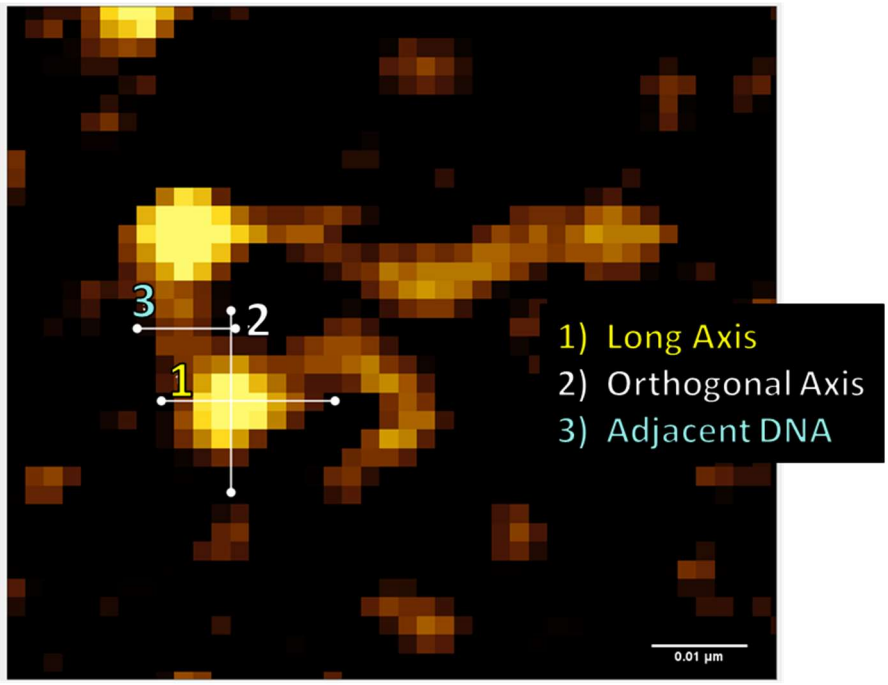
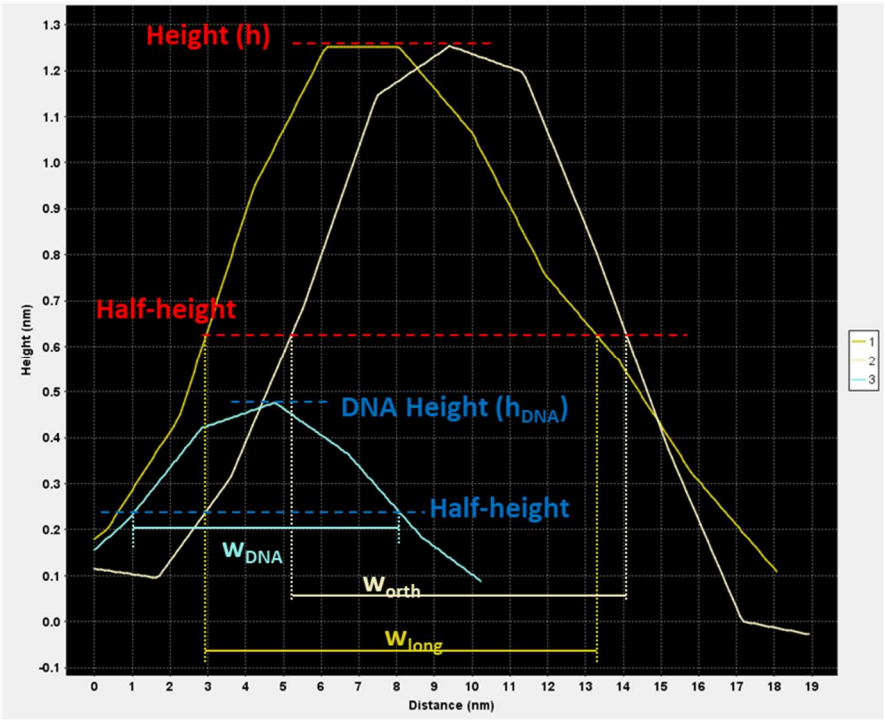
Supplemental: . Atomic force microscopy.

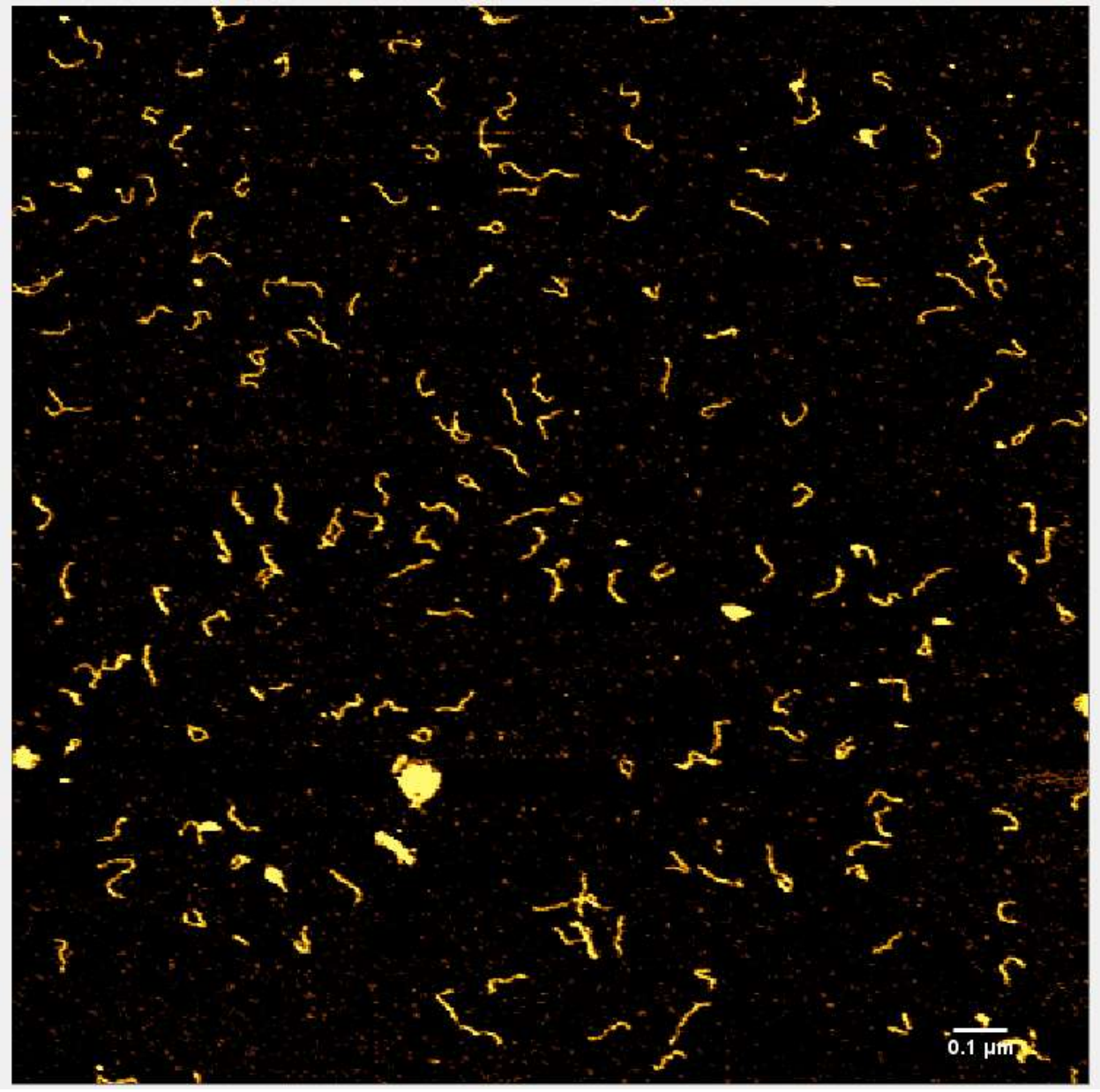
A. Parameters were measured on AFM images using the software AtomicJ (Hermanowicz, et al. 2014) as illustrated below. The colors of the numbers in the top panel correspond to the peak colors in the bottom panel. Using these parameters, peak volumes were calculated using Equations 3 and 4 (main text).



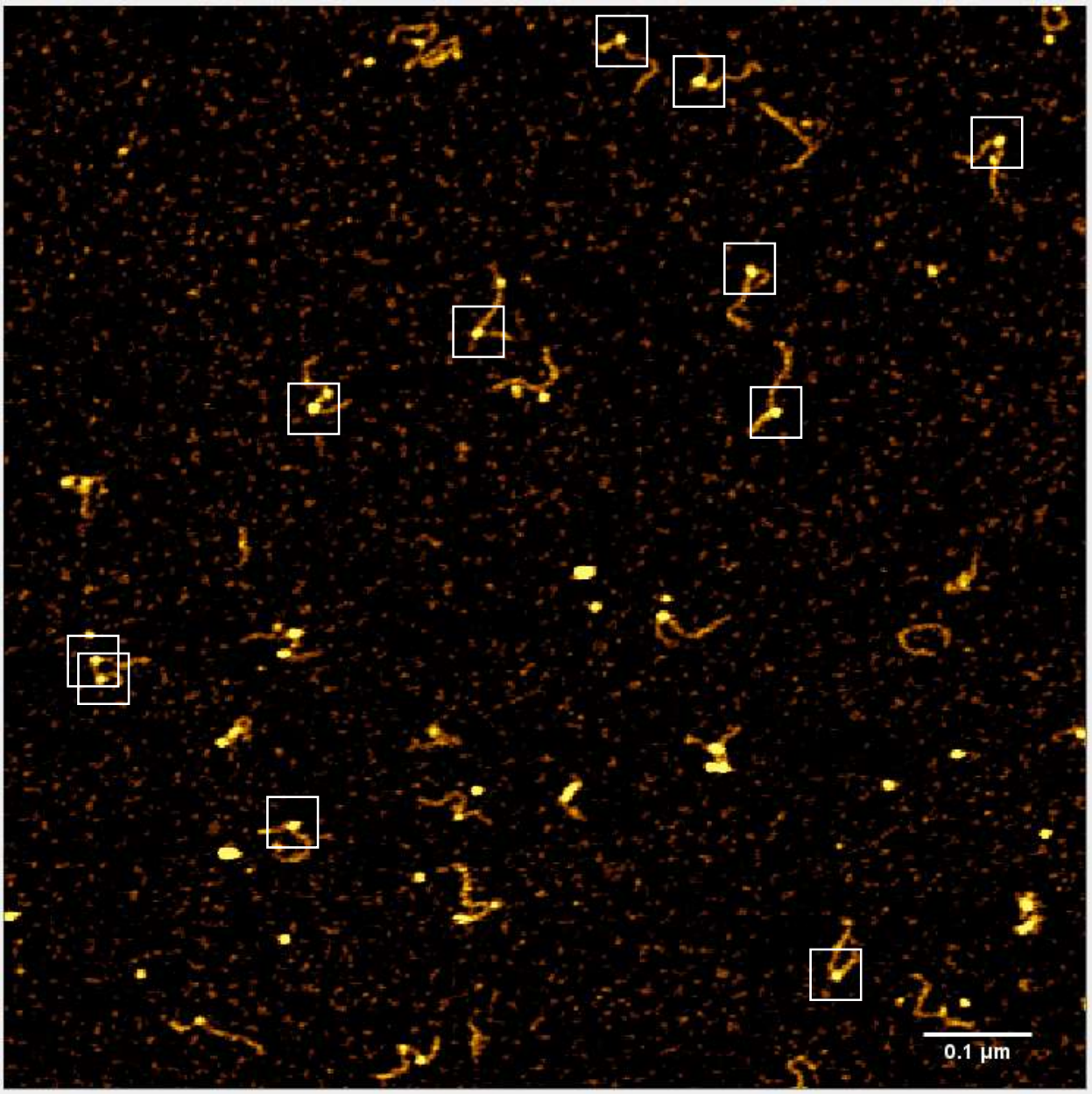


B. Full AFM images for each sample are shown on the following pages. Each image is displayed using the same depth scale from 0.2 to 1 nm; the actual maximum depth for most AFM images exceeded 3 nm. These widefield views show a 1x1 μm^2 AFM image unless otherwise indicated. Boxes (50x50 nm^2 unless otherwise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

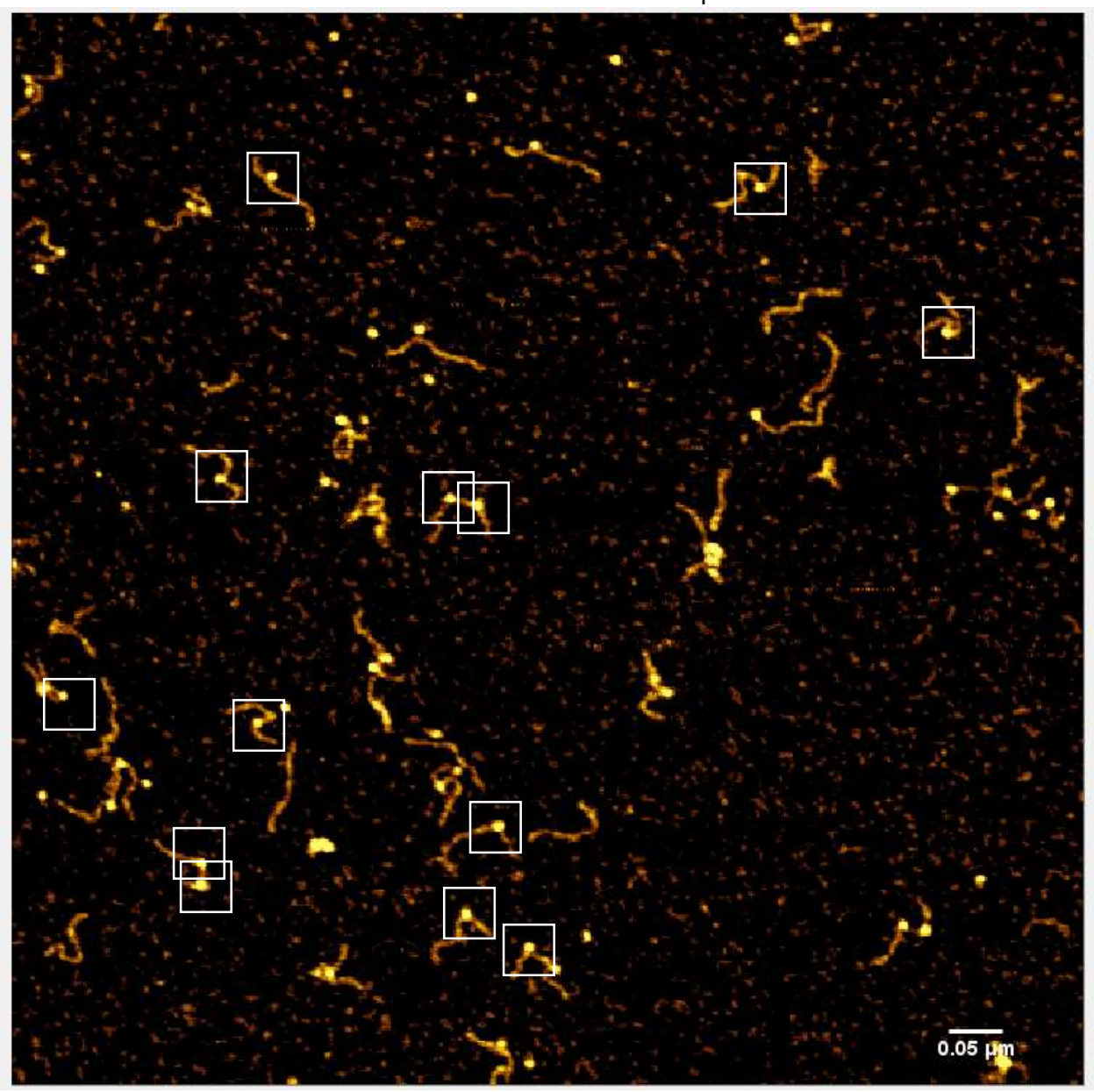
I. A sample of 10 nM DNA was diluted 1:4 (2x2 μm^2 AFM image) prior to adherence on functionalized mica. The DNA fragment (331 bp) was amplified from the cra binding region of the *fruBKA* operon.



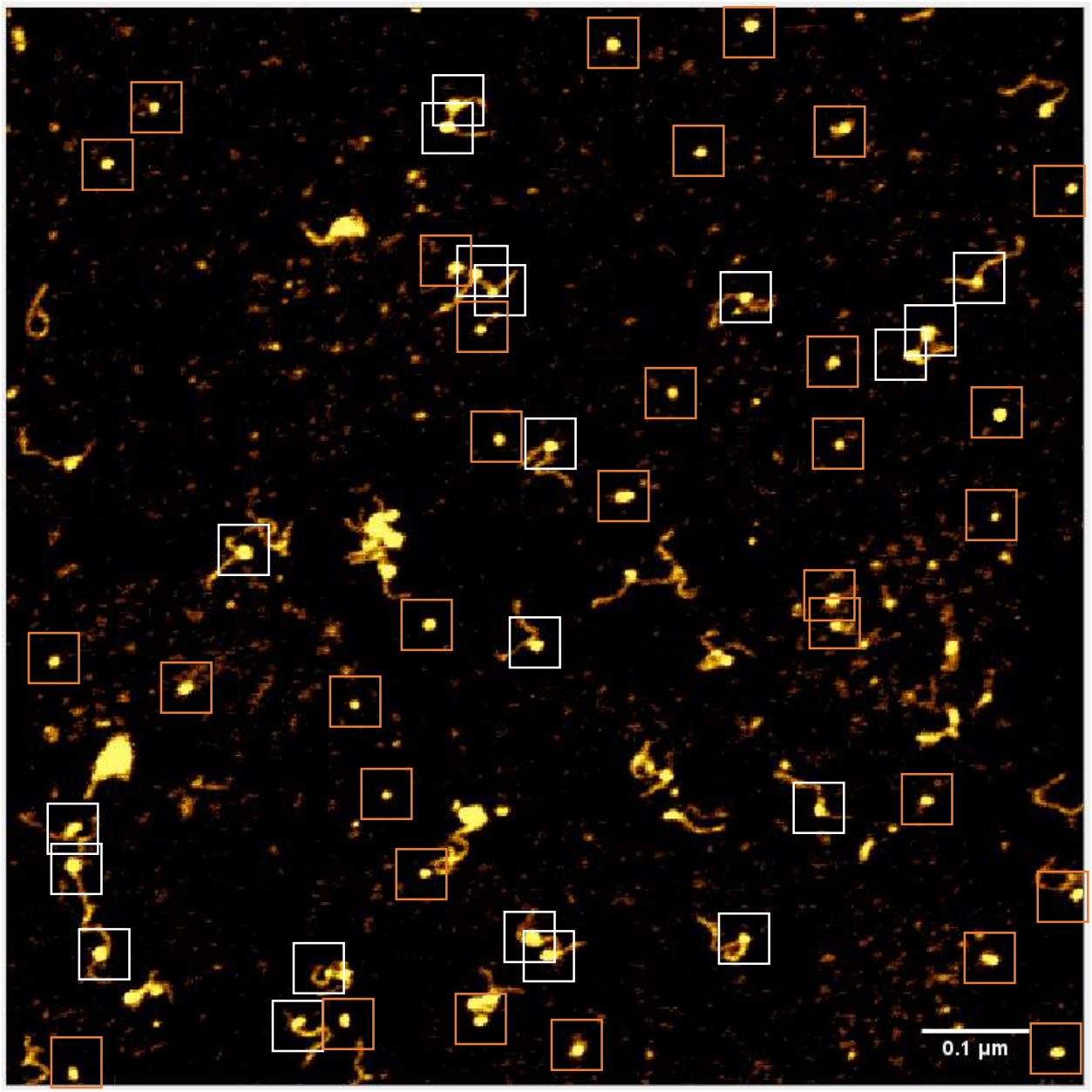
II. A sample comprising 20 nM Cra and 10 nM DNA was diluted 1:4 prior to adherence on functionalized mica. Note the additional density on the DNA that was not observed for the DNA-only sample. Each 50x50 nm^2 box indicates an analyzed protein-DNA complex.



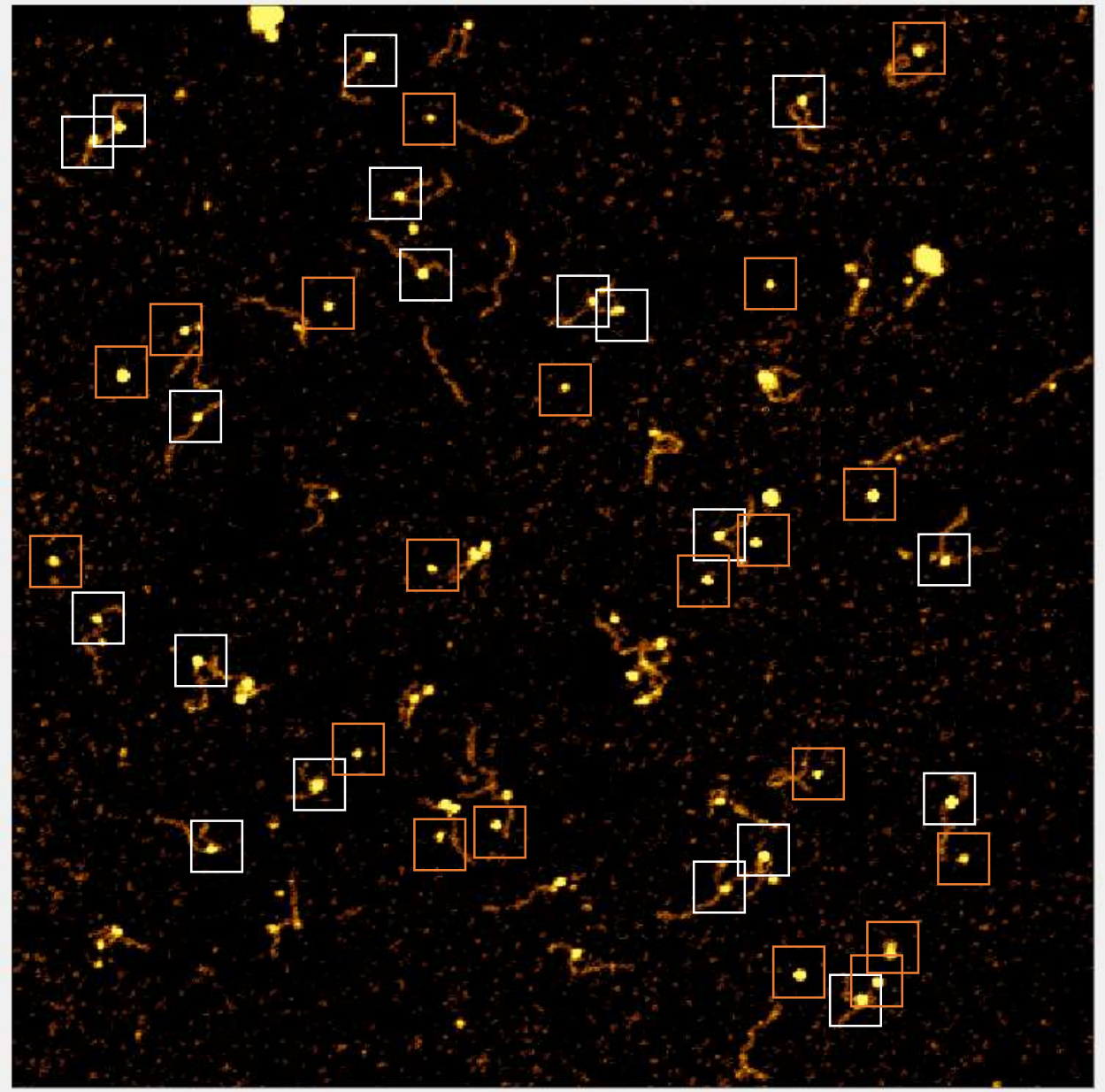
III. Additional scan site of the 20 nM Cra/10 nM DNA sample.



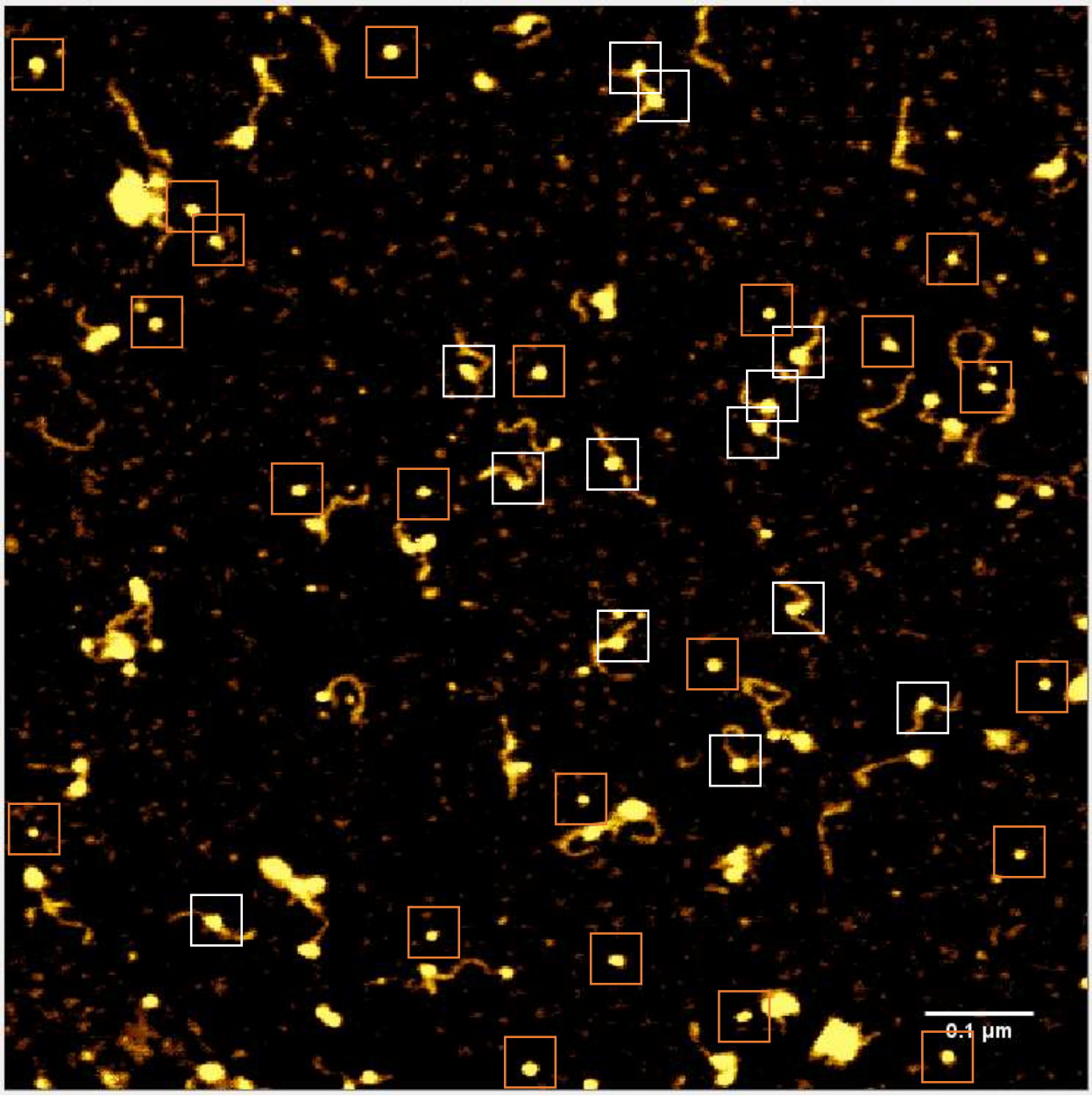
IV. A sample comprising 20 nM Cra, 20 nM FruK, and 10 nM DNA was diluted 1:4 prior to adherence on functionalized mica. As in the Cra/DNA sample, additional density was observed on the DNA (white boxes). When compared to the Cra/DNA sample, the DNA-bound density often appears larger, suggesting binding 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.



V. Additional scan site of the sample comprising 20 nM Cra, 20 nM FruK, and 10 nM DNA.



VI. 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.



VII. A sample comprising 20 nM FruK and10nM DNA was diluted 1:4 prior to adherence on functionalized mica (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.