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Supporting Material

spFRET using Alternating Excitation and FCS reveals Progressive DNA Unwrapping in Nucleosomes

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SUPPLEMENTAL MATERIALS AND METHODS

Preparation of DNA and nucleosomes

Mononucleosomes were reconstituted on a fluorescently labeled 155 (bp) DNA template containing a 601 nucleosome positioning sequence as described (1). Briefly, the template DNA was prepared by PCR and was labeled with Cy3B (donor) and ATTO647N (acceptor) by incorporation of fluorescently labeled, HPLC purified primers (IBA GmbH, Göttingen, Germany). PCR primers were as follows (modified dT or dC underlined): Cy3B labeled forward primer: 5'-TT-GGCXGGAGAATCCCGGTGCCGAGGCCGCYCAATTGGTCGTAGACAGCTCTAGCA-CCGCTTAAACGCACGZACGCGCTG-3'; ATTO647N labeled reverse primer 5'-biotin-TT-GGAZAGGATGTATATATCTGACACGTGCCTGGAGACTAGGGAGTAAYCCCCTTG-GCGGTTAAAACGXGGGGGACAGC-3'. We generated three DNA templates with this FRET pair (Förster radius R_0~ 5.5 nm) at either of the nucleosome extremes (labels at position X, or at position Z), and a position 27 basepair (bp) from one nucleosome end that we refer to as internally labeled in this work (labels at position Y), as shown in figure 1.a. In all DNA templates donor and acceptor were located ~80 bp (24 nm) apart. Nucleosomes were reconstituted by salt gradient dialysis with chicken erythrocyte histones as described (1). After reconstitution donor and acceptor were approximately 4 nm apart, resulting in efficient FRET, as confirmed by bulk fluorescence spectroscopy. Native gel electrophoresis, as described below, was used to determine the reconstitution yield, which were 70%, 90%, and 85% for reconstitutions X, Y, and Z respectively.

Sample preparation

Nucleosomes in free solution were diluted to a concentration of 100-200 pM in a buffer containing 10 mM Tris.HCl (pH 8), 0.1 mg/ml BSA, and 0.03 % NP-40. Reliable experiments could only be performed in the presence of 0.03% NP-40 anionic detergent, similar to the conditions established by Thåström et al. (2). If the NP-40 was omitted, no reproducible data could be taken and we observed a decrease in the number of bursts over time that we attributed to precipitation. For most experiments 2 mM Trolox (Sigma, Zwijndrecht, The Netherlands) was added to the buffer (3). A drop of 50 µl was placed on a glass cover slide (#1.5, Menzel, Braunschweig, Germany), and imaged as described below. Unless stated otherwise, experiments in this work were performed with nucleosomes labeled at position Y (see figure 1.a).

Nucleosomes in gel were imaged at single molecule concentration by excising the desired band from the gel. The gel slice was placed on a glass cover slide. A drop of 20 µl buffer was used to match the refractive of the gel and to prevent drying of the gel during the experiment.

Poly-acrylamide gel electrophoresis

Nucleosome reconstitutions were analyzed with 5% native poly-acrylamide gel electrophoresis (PAGE). A sample of 0.1-1 pmol was loaded on the gel (29:1 bis:acrylamide, 0.2X TB). The

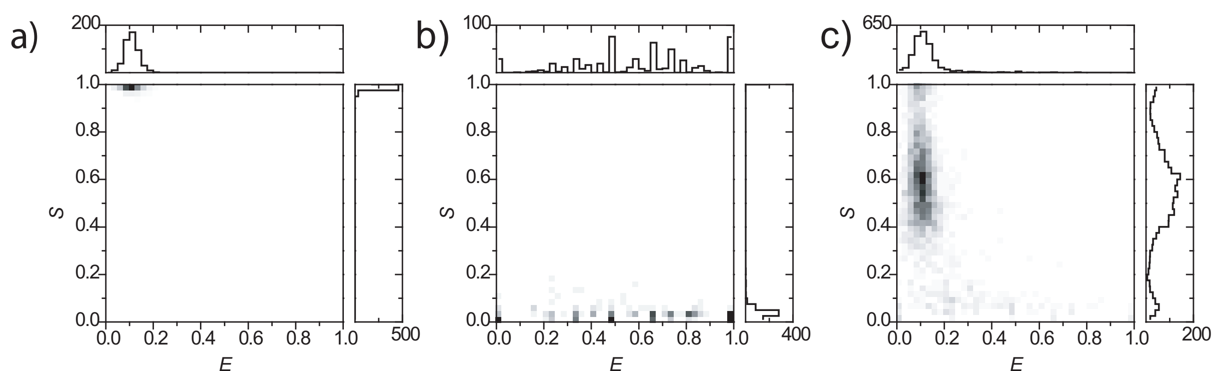

Figure S1: E,S footprint of reference samples. a) Donor-only ssDNA b) Acceptor-only ssDNA c) Donor and acceptor labeled dsDNA used in reconstitutions.

gel was run at 19 V/cm at 4 ºC for 80 min to separate nucleosomes from free DNA. The fluorescence was imaged with a gel imager (Typhoon 9400, GE, Waukesha, WI, USA).

SUPPLEMENTAL RESULTS

To characterize label stoichiometries and determine appropriate thresholds between different species, we used fluorescently labeled DNA samples that served as donor-only (D-only), acceptor-only (A-only), and doubly labeled species (D+A, no FRET). The corresponding 2D E,S-histograms revealed a single dominant population for each sample with the predicted E,S signature (Figure S1.a-c. and Table S1). Considerable D-only and A-only species were present in the doubly labeled sample, effects of photobleaching. We used these results to determine the following thresholds for the experiments on nucleosomes: D-only: $S > 0.8$, and A-only: $S < 0.2$; free DNA and unwrapped nucleosomes without FRET: $E < 0.25$; wrapped nucleosomes with significant FRET: $E > 0.25$. Since the number of molecules with E,S-values close to the thresholds was small, the number of bursts in each fraction was only marginally dependent on the position of the threshold.

Table S1: E,S signature of the dominant populations in Fig S1.a-c, and Fig 2.a.

Sample	E (mean$\pm\sigma$)	S (mean$\pm\sigma$)	fraction size
Donor-only	0.11 ± 0.06	1.00 ± 0.03	100%
Acceptor-only	0.6 ± 0.6	0.02 ± 0.02	100%
Doubly labeled DNA	0.11 ± 0.07	0.56 ± 0.28	80%
Reconstituted nucleosomes	0.63 ± 0.22	0.45 ± 0.17	78%

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