

Supporting Information

Probing Biomolecular Structures and Dynamics of Single Molecules Using In-Gel Alternating-Laser Excitation

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Abstract

This document contains the supporting information required by the primary text.

| Reagent | Source | Notes |
|-----------------------------|--------------------|------------------------------------|
| Acrylamide mixture (2.6 %C) | BioRad, CA | Acrylamide:bis-acrylamide = 37.5:1 |
| Ammonium persulfate | Fisher, UK | |
| Bovine serum albumin | Invitrogen, CA | |
| Dithiothreitol (DTT) | Fluka, Switzerland | For luminescence |
| Glycerol | Fluka, Switzerland | For luminescence |
| Glycine | Fisher, UK | |
| Heparin Sepharose | GE Healthcare, UK | |
| HEPES | Fisher, UK | |
| MgCl ₂ | Fluka, Switzerland | For luminescence |
| Potassium-L-glutamate | Sigma-Aldrich, MO | |
| TEMED | Fisher, UK | |
| Tris-base | Fluka, Switzerland | For luminescence |

Table S-1. List of buffers and gel reagents and their sources (sorted alphabetically).

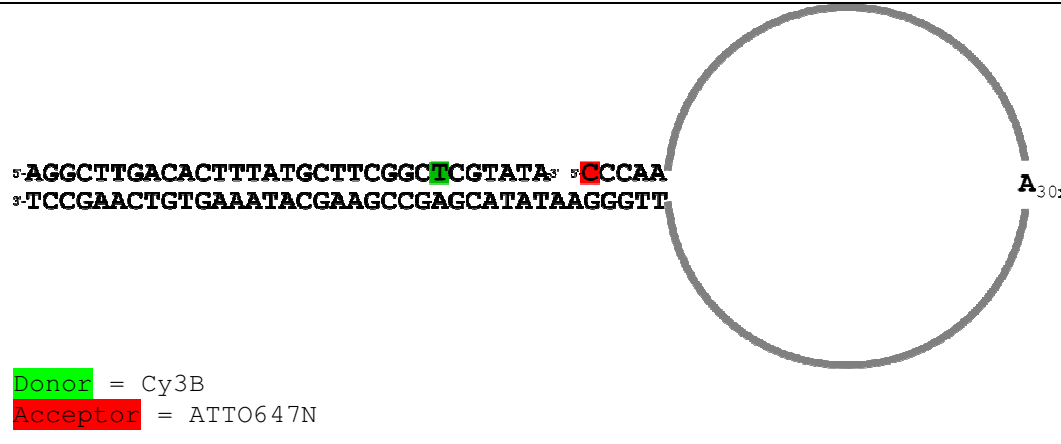
| Name | Sequences |
|--|--|
| T ₁ -Cy3B, B ₁₈ -ATTO647N | 5' - T AAATCTAAAGTAACATAAGGTAACATAACGTAAGCTCATTTCGCG-3' 3' - ATTTAGATTTTCATTGTA T TCCATTGTATTGCATTTCGAGTAAGCGC-5' Donor = Cy3B Acceptor = ATTO647N |
| T ₁ -Cy3, B ₁₈ -ATTO647N | 5' - T AAATCTAAAGTAACATAAGGTAACATAACGTAAGCTCATTTCGCG-3' 3' - ATTTAGATTTTCATTGTA T TCCATTGTATTGCATTTCGAGTAAGCGC-5' Donor = Cy3 Acceptor = ATTO647N |
| T ₁ -TMR, B ₈ -Alexa647 | 5' - T AAATCTAAAGTAACATAAGGTAACATAACGGTAAGTCCA-3' 3' - ATTTAGA T TTCATTGTATTCCATTGTATTGCCATTTCAGGT-5' Donor = TMR Acceptor = Alexa647 |
| T ₁ -TMR, B ₂₀ -Cy5 | 5' - T ACTACTGGGCCATCGTGAC-3' 3' - TTGATGACCCGGTAGCACT T -5' Donor = TMR Acceptor = Cy5 |
| T ₂₅ -Cy3B, B ₅₄ -Alexa647 | 5' - AGGCTTGACACTTTATGCTTCGGC T CGTATAATGTGTGGAATTGTGAGAGCGGA-3' 3' - TCCGAAGTGTGAAATACGAAGCCGAGCATATTACACACCTTAACACTCTCGCC T -5' Donor = Cy3B Acceptor = Alexa647 |
| Hairpin | <div style="text-align: center;">  <p> 5'-AGGCTTGACACTTTATGCTTCGGCTCGTATA-3' A_{30x} 3'-TCCGAAGTGTGAAATACGAAGCCGAGCATATAAGGGTT-5' </p> <p> Donor = Cy3B Acceptor = ATTO647N </p> </div> |

Table S-2. List of DNA sequences used in the primary paper. The nomenclature for linear DNA is “T_{X-D},B_{Y-A}”, where *T* is top strand, *B* is bottom strand, *X* & *Y* indicate the labeling positions as the number of bases counted from the 5' end of the top strand, and *D* & *A* indicate the fluorophores. The bases labeled with the FRET donors are highlighted in green, while those labeled with the FRET acceptors are highlighted in red.

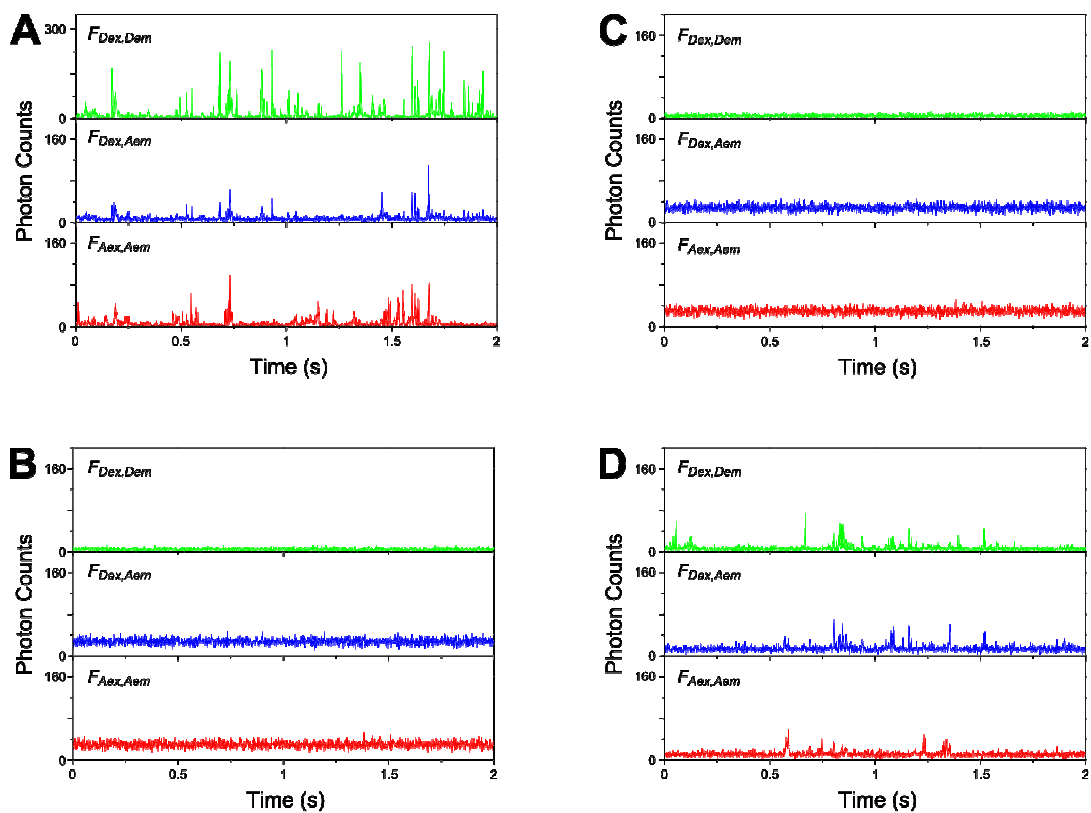


Figure S-1. Confocal detections of hairpin molecules co-polymerized inside 6% PA. Fluorescence time traces (1-ms bins; green: $F_{Dex, Dem}$; blue: $F_{Dex, Aem}$; red: $F_{Aex, Aem}$) of (A) 100 pM labeled hairpin measured in solution, (B) blank 6% PA, and (C) 1 nM hairpin measured in 6% PA. The traces in (D) were obtained from sample (C), but measured very close ($< 3\mu\text{m}$) from the coverslip surface. The absence of fluorescence bursts in gel (indicating complete immobilization of molecules) and the low intensity even at high concentration of labeled molecules illustrate the perturbative effects of this approach compared to in-gel ALEX (compare to Fig. 2 in main text).

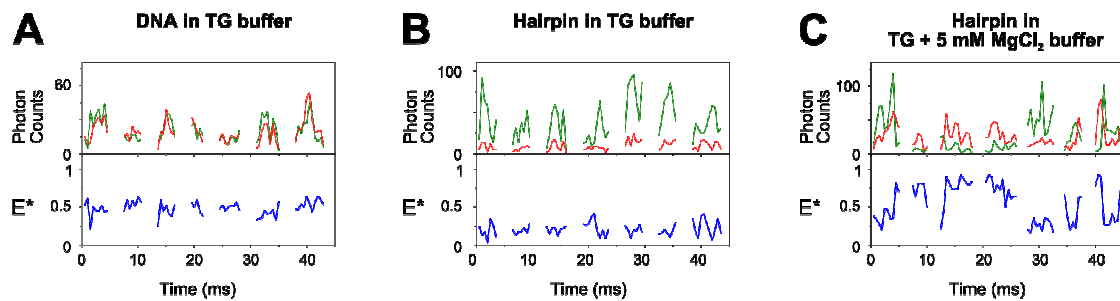


Figure S-2. Representative in-solution time-traces of (A) double-stranded DNA (T_{1-Cy3B} , $B_{18-ATTO647N}$), (B) DNA hairpin in the absence of $MgCl_2$, and (C) DNA hairpin in the presence of 5 mM $MgCl_2$. The samples in panels A and B were measured in TG buffer, while the hairpin in panel C was measured in TG buffer containing 5 mM $MgCl_2$. Burst selection criteria: $L=60$, $M=10$, $T=1$ ms, $S>0.5$, burst length >1.8 ms; per-0.5ms bin filter: $S>0.5$, $F_{Dex}>10$; for details, see *Methods: Data analysis* in main text. The upper panels show the photon counts due to ALEX partitioned into 0.5-ms time bins (green, $F_{Dex, Dem}$; red, $F_{Dex, Aem}$; $F_{Aex, Aem}$, not shown). The lower panels show the E^* values for each 0.5-ms bin. The minimum burst length for in-solution bursts is chosen such that it selects the same fraction of molecules as in 6% PA. Notice that the bursts in C are much shorter compared to those collected in 6% PA (Fig. 5A, main text). The short burst duration compared to the timescale of dynamics makes it difficult to draw conclusions regarding dynamics solely from in-solution time-traces.