Supporting Information

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

Yusdi Santoso[*,1], *Achillefs N. Kapanidis*[1]

[1]Department of Physics and Biological Physics Research Group, Clarendon Laboratory,

University of Oxford, Parks Road, Oxford, OX1 3PU, United Kingdom

* Corresponding authors: y.santoso1@physics.ox.ac.uk (Y.S.), a.kapanidis1@physics.ox.ac.uk (A.N.K.), Fax: +44-1865-282208 (A.N.K.).

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).

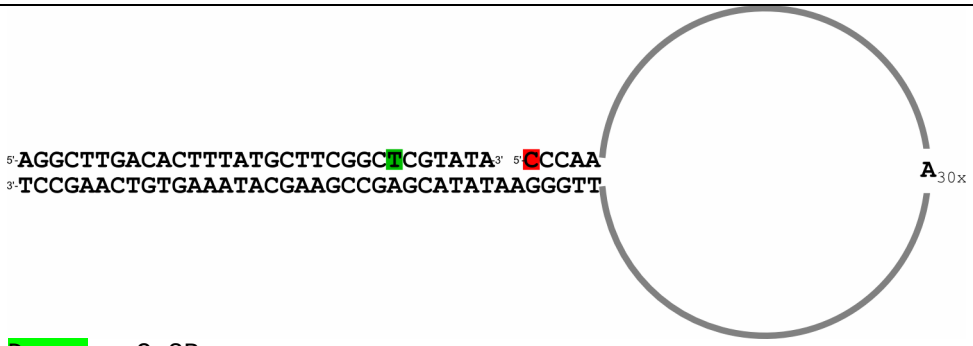
Name	Sequences
$T_{1\text{-Cy3B}},B_{18\text{-ATTO647N}}$	5′-TAAATCTAAAGTAACATAAGGTAACATAACGTAAGCTCATTCGCG-3′ 3′-ATTTAGATTTCATTGTATTCCATTGTATTGCATTCGAGTAAGCGC-5′ Donor = Cy3B Acceptor = ATTO647N
$T_{1\text{-Cy3}},B_{18\text{-ATTO647N}}$	5′-TAAATCTAAAGTAACATAAGGTAACATAACGTAAGCTCATTCGCG-3′ 3′-ATTTAGATTTCATTGTATTCCATTGTATTGCATTCGAGTAAGCGC-5′ Donor = Cy3 Acceptor = ATTO647N
$T_{1\text{-TMR}},B_{8\text{-Alexa647}}$	5′-TAAATCTAAAGTAACATAAGGTAACATAACGGTAAGTCCA-3′ 3′-ATTTAGATTTCATTGTATTCCATTGTATTGCCATTCAGGT-5′ Donor = TMR Acceptor = Alexa647
$T_{1\text{-TMR}},B_{20\text{-Cy5}}$	5′-AACTACTGGGCCATCGTGAC-3′ 3′-TTGATGACCCGGTAGCACTG-5′ Donor = TMR Acceptor = Cy5
$T_{25\text{-Cy3B}},B_{54\text{-Alexa647}}$	5′- AGGCTTGACACTTTATGCTTCGGCTCGTATAATGTGTGGAATTGTGAGAGCGGA-3′ 3′- TCCGAACTGTGAAATACGAAGCCGAGCATATTACACACCTTAACACTCTCGCCT-5′ Donor = Cy3B Acceptor = Alexa647
Hairpin	 Donor = Cy3B Acceptor = ATTO647N

Table S-2. List of DNA sequences used in the primary paper. The nomenclature for linear DNA is "$T_{X\text{-}D},B_{Y\text{-}A}$", 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.

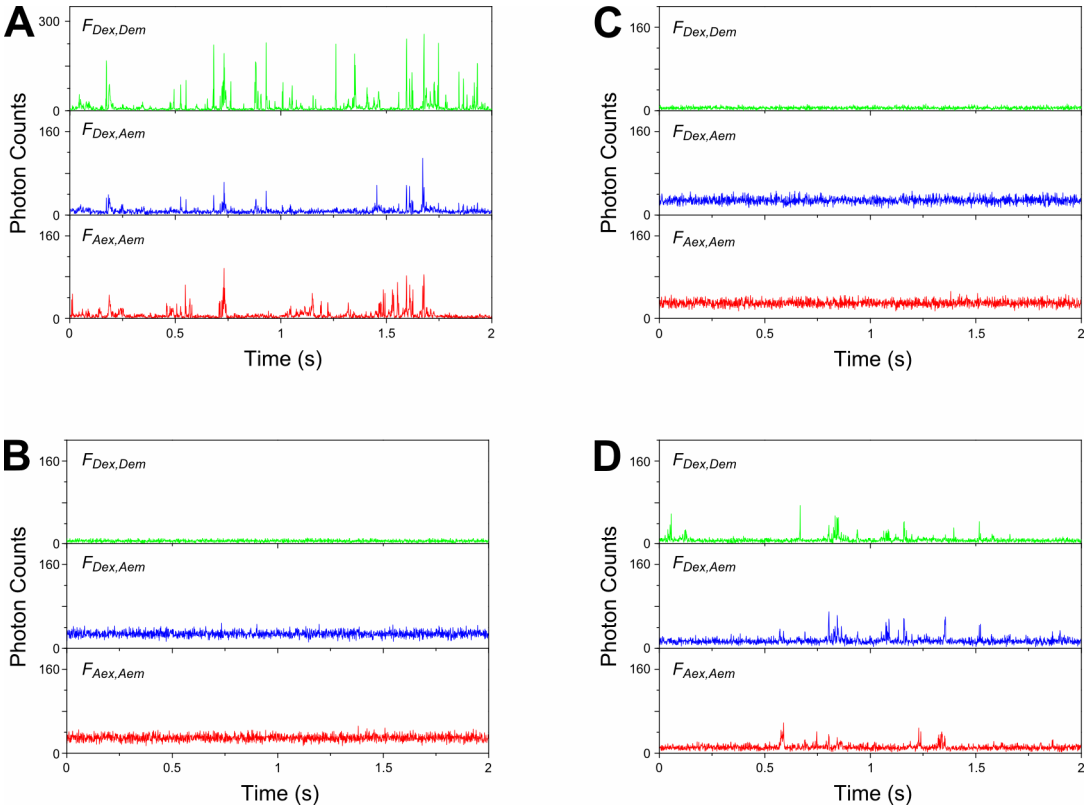


Figure S-1. Confocal detections of hairpin molecules co-polymerized inside 6% PA. Fluorescence time traces (1-ms bins; green: $F_{Dex,Dem}$; blue: $F_{Dex,Aem}$; red: $F_{Aex,Aem}$) of (A) 100 pM labeled hairpin measured in solution, (B) blank 6% PA, and (C) 1 nM hairpin measured in 6% PA. The traces in (D) were obtained from sample (C), but measured very close (< 3μm) 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).

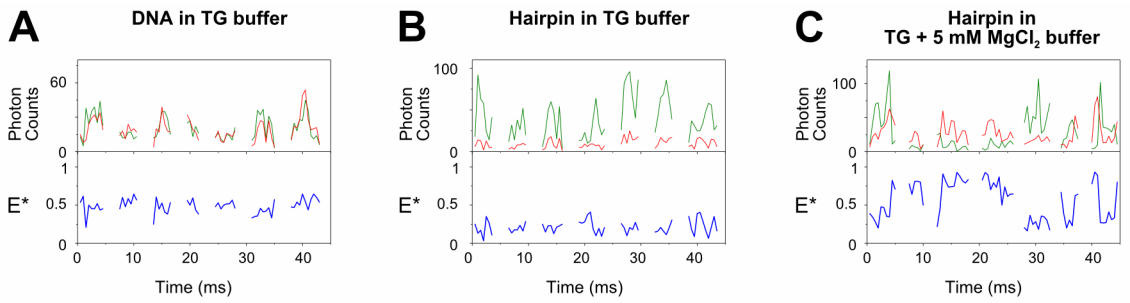


Figure S-2. Representative in-solution time traces of (A) double-stranded DNA ($T_{1\text{-Cy3B}}$,$B_{18\text{-ATTO647N}}$), (B) DNA hairpin in the absence of $MgCl_2$, and (C) DNA hairpin with 5 mM $MgCl_2$. The samples in A-B were measured in TG buffer, while the hairpin in C was measured in TG buffer 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; see *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 is chosen such that the same fraction of molecules is selected in solution as in 6% PA (minimum burst length in solution : τ_D in solution = minimum burst length in 6% PA : τ_D in 6% PA). Notice that the bursts in C are much shorter compared to those measured in 6% PA (see Fig. 5A in the main text). The short burst length compared to the timescale of dynamics makes it impossible to draw reliable inference about the dynamics solely from in-solution time traces data.