Accurate Molecular Sizing of Single Molecules Limited by Rhodamine Photoblinking

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Abstract

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Introduction

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$$D_{coll} = \frac{D_f + \frac{[S]^2}{K_D S_T} D_S}{1 + \frac{[S]^2}{K_D S_T}}, D_{sm} = \frac{D_f + \frac{[S]}{K_D} D_S}{1 + \frac{[S]}{K_D}}, \quad (1)$$

Materials and Methods

Preparation of DNA Holliday Junctions

Holliday Junction Preparation Four oligonucleotides labeled at the 5-prime end by the fluorescent dye Alexa Fluor 488 and with the correct sequence to anneal to form a Holliday Junction **??** were purchased (atdbio), along wih equivalent unlabelled strands (Sigma Life Science). Their sequences are given in Table 1. The four strands required to generate a Holliday junction were combined in Tris buffer (10 mM, pH 8.0, 50 mM NaCl), to give a final total DNA concentration of 5 μM in a total volume of 20 μL, using an equimolar concentration of the four DNA strands. The single-stranded DNAs were annealed into the Holliday Junction by heating to 95$\circ$C for 10 minutes, followed by slow cooling overnight to 25$\circ$C.

Table 1. DNA sequences of the four arms of the Holliday Junction

Arm	Sequence
B	CCCTAGCAAGCCGCTGCTACGG**5**
H	CCGTAGCAGCGAGAGCGGTGGG**5**
R	CCCACCGCTCTTCTCAACTGGG**5**
X	CCCAGTTGAGAGCTTGCTAGGG**5**

Each sequence is shown in the 3' - 5' direction/ **5** is a maleimide linkage to Alexa Fluor 488, present in the fluorescently labelled DNA strands, but absent in their unlabelled partners.

Holliday Junction Purification After annealing, the Holliday Junctionss were purified from the reaction mixture using gel electrophoresis on an 8% TBE acrylamide gel in TBE buffer (Novex, Life Technologies Ltd.). The complete Holliday Junction was identified by visualising the bands using low intensity (PMT 300V) excitation at 526 nm (XXX CHECK THIS WIH VLADAS) and comparison with a 10 bp DNA ladder. The band corresponding to the complete Holliday Junction was cut from the gel and eluted into 200 μL of Tris buffer by passive difusion overnight. Holliday Junctions were then stored in the dark at 4$\circ$C until required.

Simulations

Confocal Measurements of Holliday Junctions

Photobleaching Analysis of Holliday Junctions

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1. react
2. diffuse free particles
3. increment time by dt and go to 1

Results and Discussion

Confocal Measurement of the Holliday Junctions

Understanding the Photon Emission Distribution Through Mathematical Modelling

Photobleaching Analysis of the Holliday Junctions Identifies Sources of Heterogeneity

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Table 2. Table caption Nulla mi mi, venenatis sed ipsum varius, volutpat euismod diam.

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*cell*1*row*2	cell2 row 2	cell3 row 2	cell4 row 2	cell5 row 2	cell6 row 2	cell7 row 2	cell8 row 2
*cell*1*row*3	cell2 row 3	cell3 row 3	cell4 row 3	cell5 row 3	cell6 row 3	cell7 row 3	cell8 row 3

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Discussion

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Supporting Information

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References

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