

Supporting Information

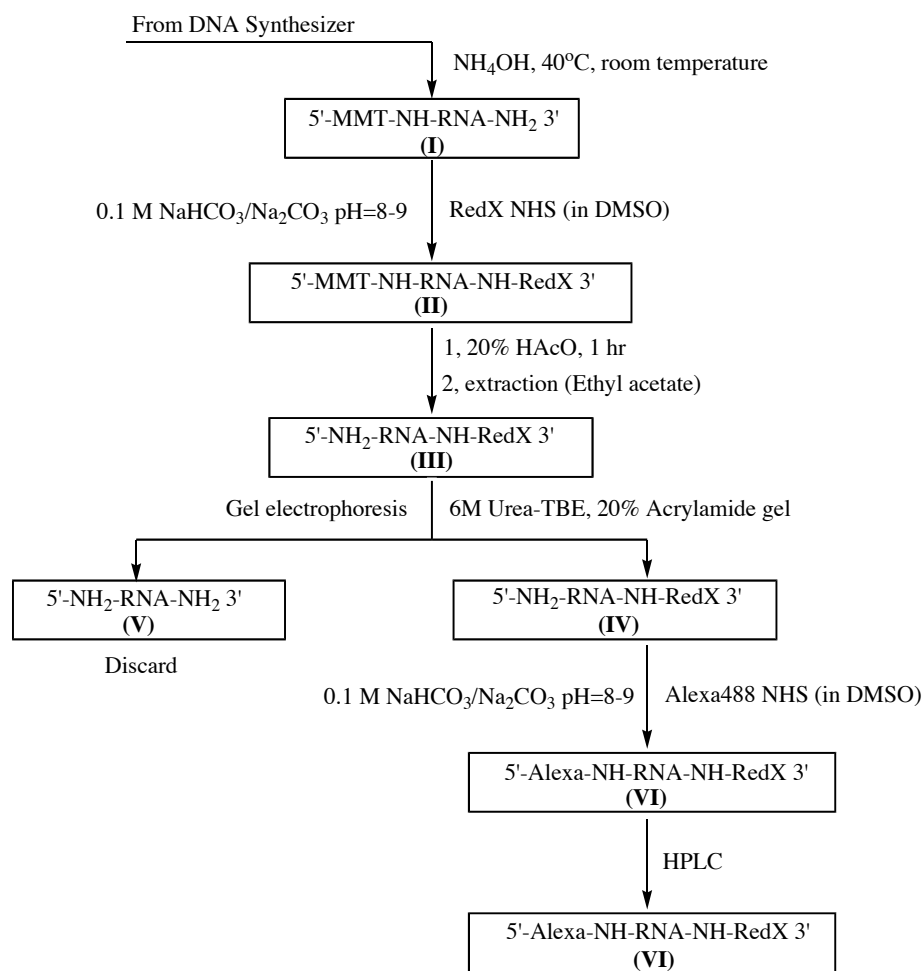
Spectroscopic investigation of a FRET Molecular Beacon containing two fluorophores for probing DNA/RNA sequences.

Steffen Jockusch,^a Angel A. Martí,^a Nicholas J. Turro,^{a,b,*} Zengmin Li,^{b,c} Xiaoxu Li,^c Jingyue Ju,^{b,c} Nathan Stevens,^d and Daniel L. Akins^d

Departments of ^aChemistry and ^bChemical Engineering, Columbia University, New York, NY, 10027.

^cColumbia Genome Center, Columbia University College of Physicians and Surgeons, New York, NY, 10032.

^dDepartment of Chemistry, City College, City University of New York, NY 10031.



Scheme S1: Schematic representation of the synthesis of Alexa-MB-RedX.

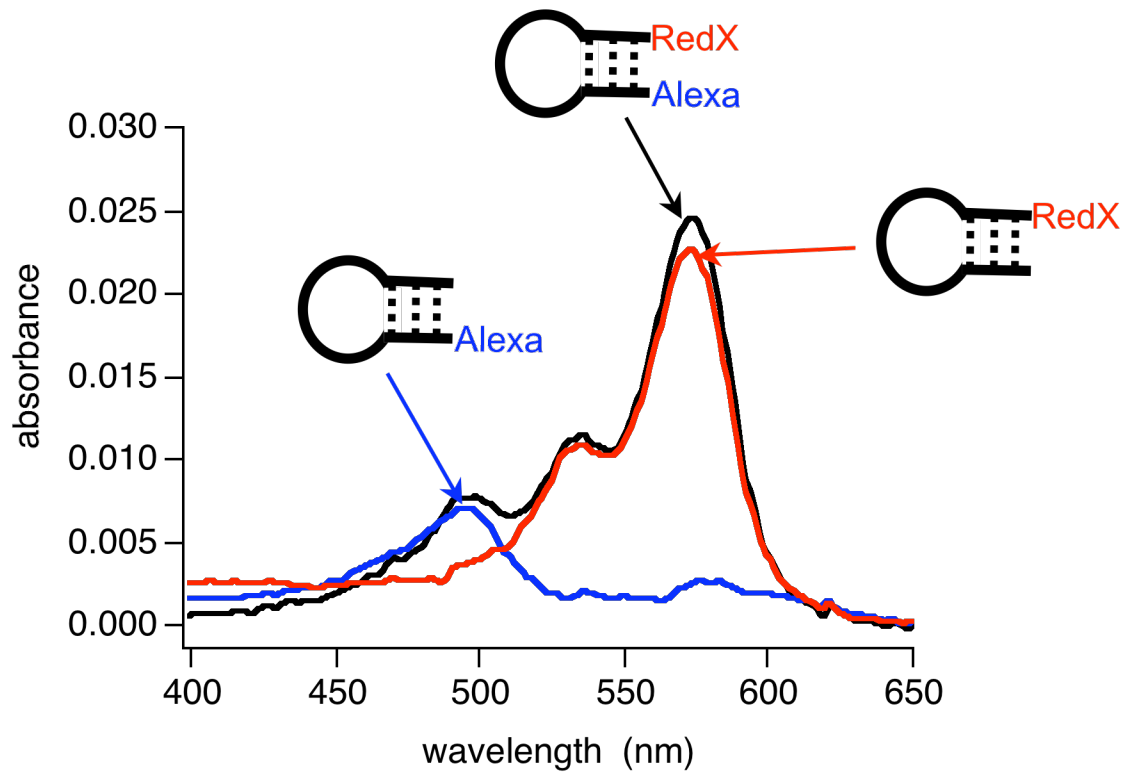


Figure S1: Absorbance spectra (smoothed) of Alexa-MB-RedX (black) Alexa-MB (blue) and MB-RedX (red) ($1 \mu\text{M}$) in aqueous buffer solutions (20 mM Tris, 60 mM MgCl_2), 500 mM NaCl; pH = 7.0) at a 4 mm path length.

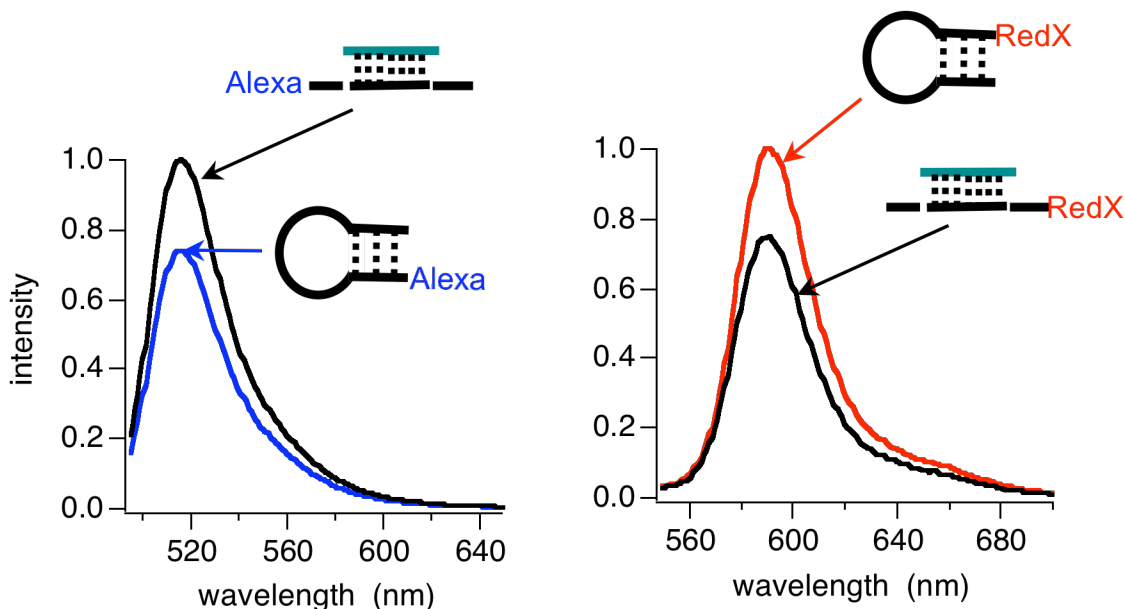


Figure S2: Fluorescence spectra after excitation of Alexa-MB (left) and MB-RedX (right) (1 μ M) in aqueous buffer solutions (20 mM Tris, 60 mM $MgCl_2$), 500 mM NaCl; pH = 7.0) at 488 nm in the absence (blue or red spectra) and presence (black spectra) of target DNA (10 μ M).

The fluorescence intensity of the stem-open form (presence of target) increased approximately 35 % compared to the stem-closed form (Figure S2, left). This quenching effect of Alexa in the stem-closed form is probably caused by guanosine residues. Because Alexa is covalently attached to dC, in the stem-closed form dG is in close proximity due to hybridization with dC (Scheme 2) and probably causing some quenching. In the stem-open form the guanosine residue is separated from Alexa and an approximately 35 % stronger fluorescence is observed (Figure S2, left). Similar quenching effects have been reported for fluorescent dye linked oligonucleotides [Ref. 19-21]. An opposite fluorescence quenching effect has been observed for MB-RedX, where the fluorescence of RedX decreased by approximately 25 % upon hybridization with the target (Figure S2, right) This fluorescence decrease could be caused by making the guanosine residue sterically more accessible to RedX excited states in the stem-open form.

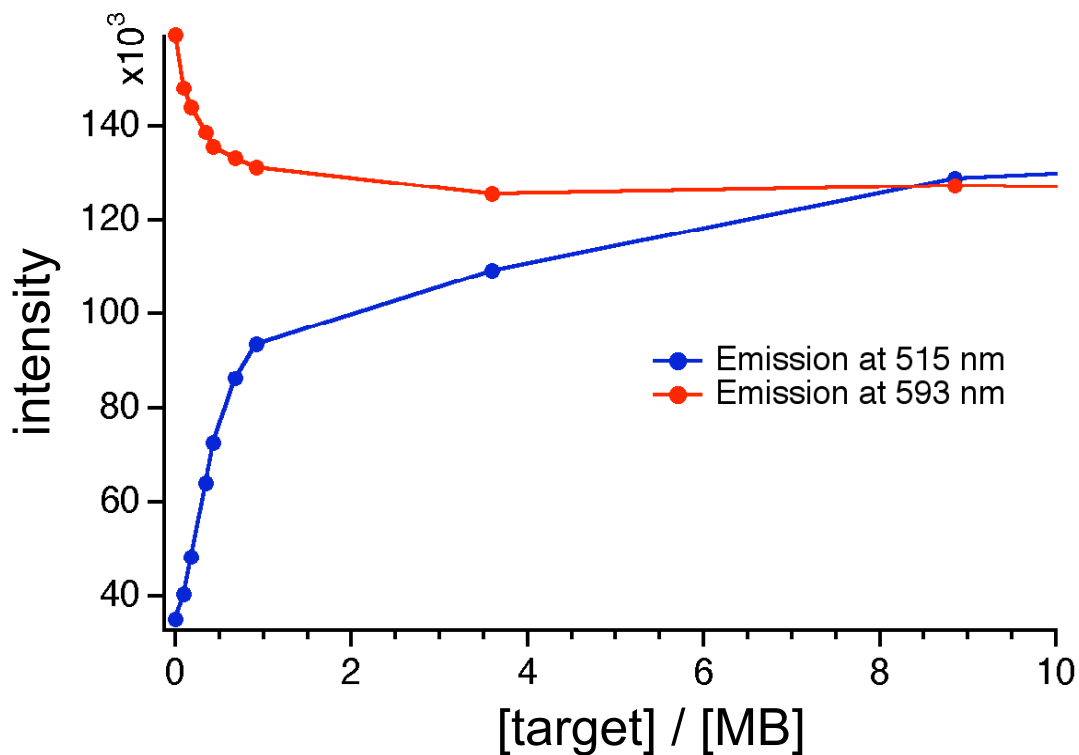


Figure S3: Fluorescence intensity of Alexa-MB-RedX at 515 nm (blue) and 593 nm (red) in the presence of different amounts of target DNA. $[MB] = 0.22 \mu M$, 10 min equilibration time between additions.

Figure S3 demonstrates that the MB opens efficiently in the presence of target DNA. At a ratio $[MB] : [target]$ of 1 : 1, more than half of the MB opened to hybridize with the target DNA.

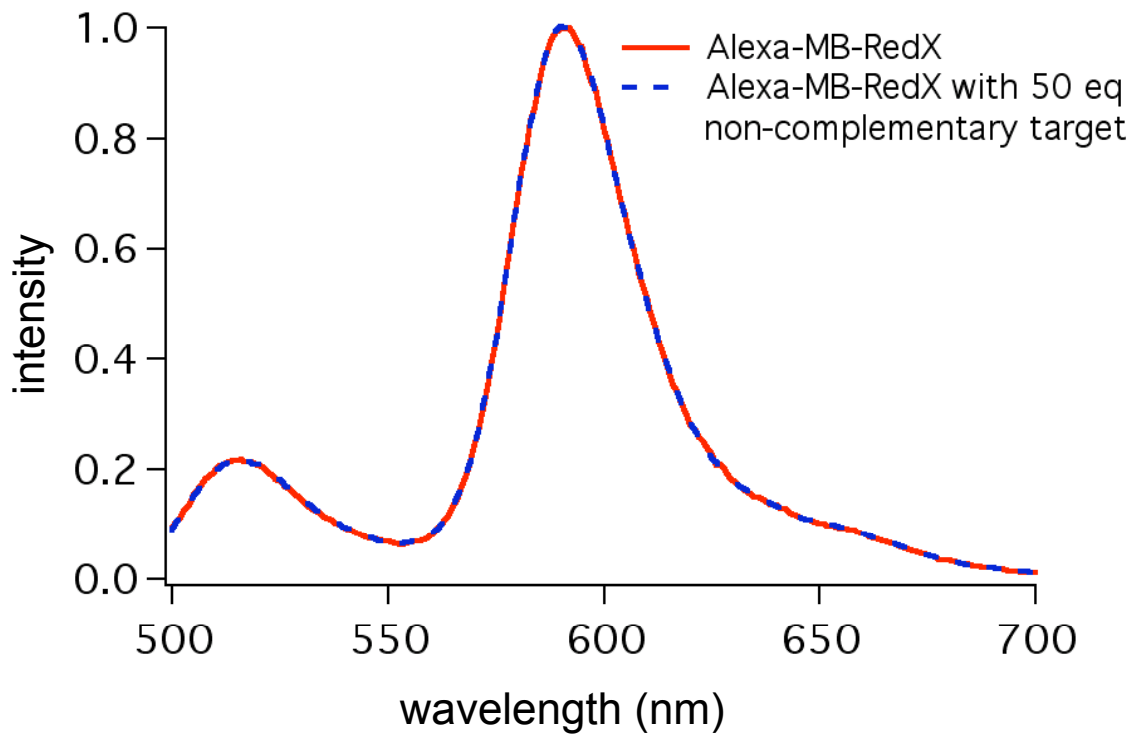


Figure S4: Fluorescence spectra of Alexa-MB-Rox in the absence (red) and presence (blue) of 50 equivalents of non-complementary target. $[MB] = 0.22 \mu\text{M}$, $[\text{target}] = 1.1 \mu\text{M}$, 2h equilibration time between addition of target, non-complementary target sequence: 5'-CAT AGG TCT TAA CTT-3', $\lambda_{\text{ex}} = 488 \text{ nm}$.

Because the fluorescence signature of the molecular beacon, Alexa-MB-RedX, does not change after addition of a non-complementary DNA sequence, it can be concluded that the MB remains in the stem-closed configuration in the presence of an excess of DNA, which is not complimentary to the loop sequence.