

Supplementary Data

Dual-color control of nucleotide polymerization sensed by fluorescence
actuator

Madalena M. Reimão-Pinto^{†[a]}, Ana Cordeiro^{†[a,b]}, Carina Almeida^[a], André V.
Pinheiro^[a,b], Artur Moro^[b], João C. Lima^{*[b]} and Pedro V. Baptista^{*[a]}

^[a] CIGMH, Departamento Ciências da Vida, Faculdade de Ciências e Tecnologia,
Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

^[b] REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia,
Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

*Corresponding author. J.C. Lima Email: lima@fct.unl.pt; P.V. Baptista Email:
pmvb@fct.unl.pt; Phone/fax: +351 212948530

[†]These authors contributed equally to the work.

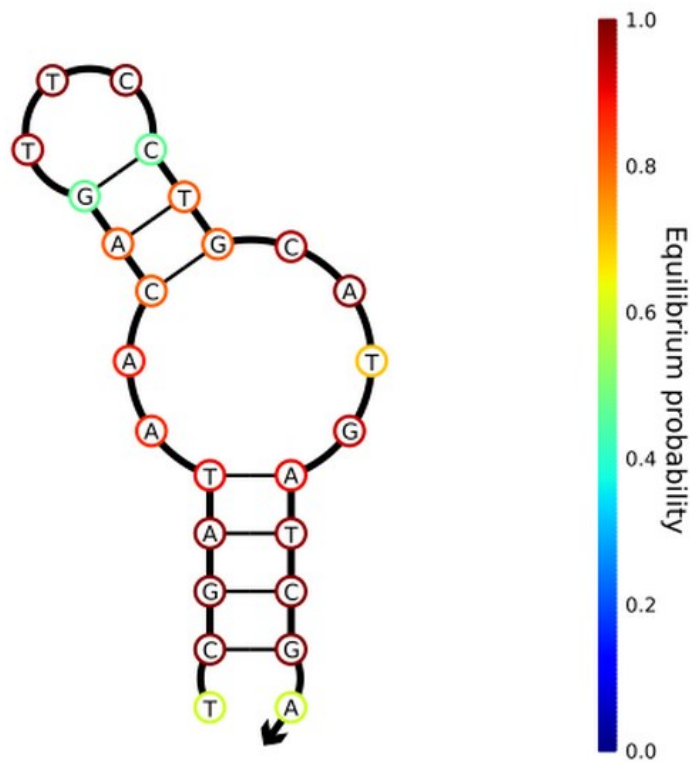


Fig. S1 Representation of the molecular beacon secondary structure. Each color represents the equilibrium probability for different nucleotide interactions. At 20 °C, the free energy for the formation of secondary structure is $-18.54 \text{ kJ}\cdot\text{mol}^{-1}$.

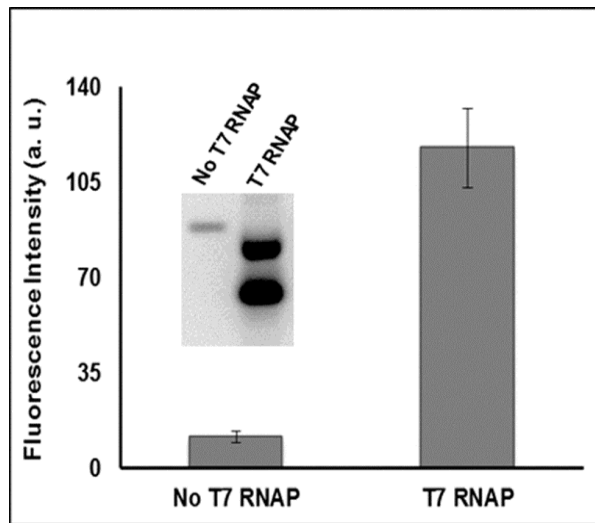


Fig. S2 Calibration of the molecular beacon with the target transcription product. In the absence of T7 RNA polymerase, transcription does not occur and no RNA product is formed. Therefore, the beacon maintains its closed conformation and no fluorescence signal is detected. In the presence of T7 RNA polymerase, the transcription product is synthesized that causes the beacon to open upon hybridization to emerging target RNA, resulting in an increased fluorescence signal. Average and standard deviation values were obtained from 5 independent replicates.

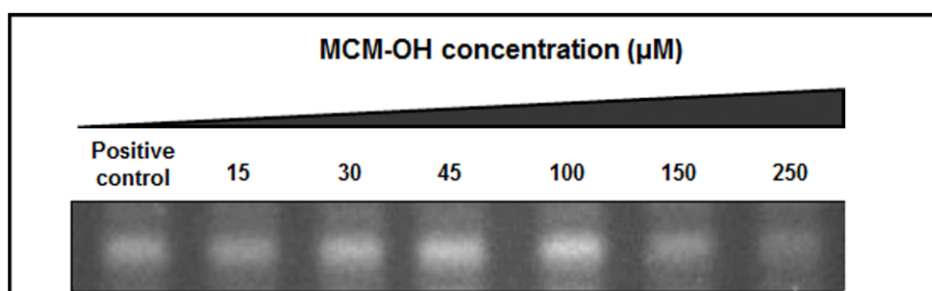


Fig. S3 MCM-OH inhibition assay. Agarose gel electrophoresis of T7 RNA polymerase *in vitro* transcription reaction with 2 mM of CTP, GTP, UTP and ATP in the presence of increasing MCM-OH concentrations. Positive control refers to the reaction in the absence of MCM-OH. No inhibitory effect of transcription is observed for MCM-OH concentrations of and under 100 μM .