Supplementary Data

Yan Shan Ang and Lin Yue Lanry Yung*

Department of Chemical and Biomolecular Engineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260

*Corresponding author (cheyly@nus.edu.sg)

Table S1. List of all DNA sequences used in this study.

Strand	Sequence
Optimization of molecular switch design	
Design i	GGT TGG TGT GGT TGG ACA CCA ACC AGT CTA GGA TTC GGC GTG
Design ii	GGT TGG TGT GGT TGG CAA CCA GTC TAG GAT TCG GCG TG
Design iii	CTG GTT GGT GTG GTT GGC AAC CAG TCT AGG ATT CGG CGT G
Design iv	GAC TGG TTG GTG TGG TTG GCA ACC AGT CTA GGA TTC GGC GTG
Design v	CAA CCA GTC TAG GAT TCG GCG TGC TAG ACT GGT TGG TGT GGT TGG
Module kinetics – hybridization chain reaction	
Hairpin 1 (HP1)	TTA ACC CAC GCC GAA TCC TAG ACT CAA AGT AGT CTA GGA TTC GGC GTG
Hairpin 2 (HP2)	AGT CTA GGA TTC GGC GTG GGT TAA CAC GCC GAA TCC TAG ACT ACT TTG
c* b*	AGT CTA GGA TTC GGC GTG GGT TAA
Module kinetics – signal transduction	
Protector (P)	TTA ACC CAC GCC GAA TCC TAG ACT GGT TG
t* c*	ACA CCA ACC AGT CTA GGA TTC GGC GTG
Module kinetics – α-thrombin recognition	
Initiator 1 (I1)	Refer to sequence of molecular switch design iii.
Initiator 2 (I2)	AGT CTA GGA TTC GGC GTG GGT TAA TTT TTT TTT TTT TTT AGT CCG TGG TAG GGC AGG TTG GGG TGA CT
Formation of localized signal	
15T	Refer to sequence of I2.
5T	AGT CTA GGA TTC GGC GTG GGT TAA TTT TTA GTC CGT GGT AGG GCA GGT TGG GGT GAC T
Unbound (noApt)	AGT CTA GGA TTC GGC GTG GGT TAA AGT CCG TGG TAG GGC AGG TTG GGG TGA C

Table S2. List of domain sequences involved in the DNA circuit design.

Domain	Description	Length (nt)	Sequence
a1	Part of binding sequence to the fibrinogen-recognition exosite	10	GTG TGG TTG G
a2	Binds to the heparin binding exosite	29	AGT CCG TGG TAG GGC AGG TTG GGG TGA CT
b	Alternating toehold of HCR	6	TTA ACC
c	Stem of HCR hairpins	18	CAC GCC GAA TCC TAG ACT
d	Alternating toehold of HCR	6	CAA AGT
e	Additional lock	2	CT
s	Distance modulator	15	TTT TTT TTT TTT TTT
t	Sequestered toehold	5	GGT TG

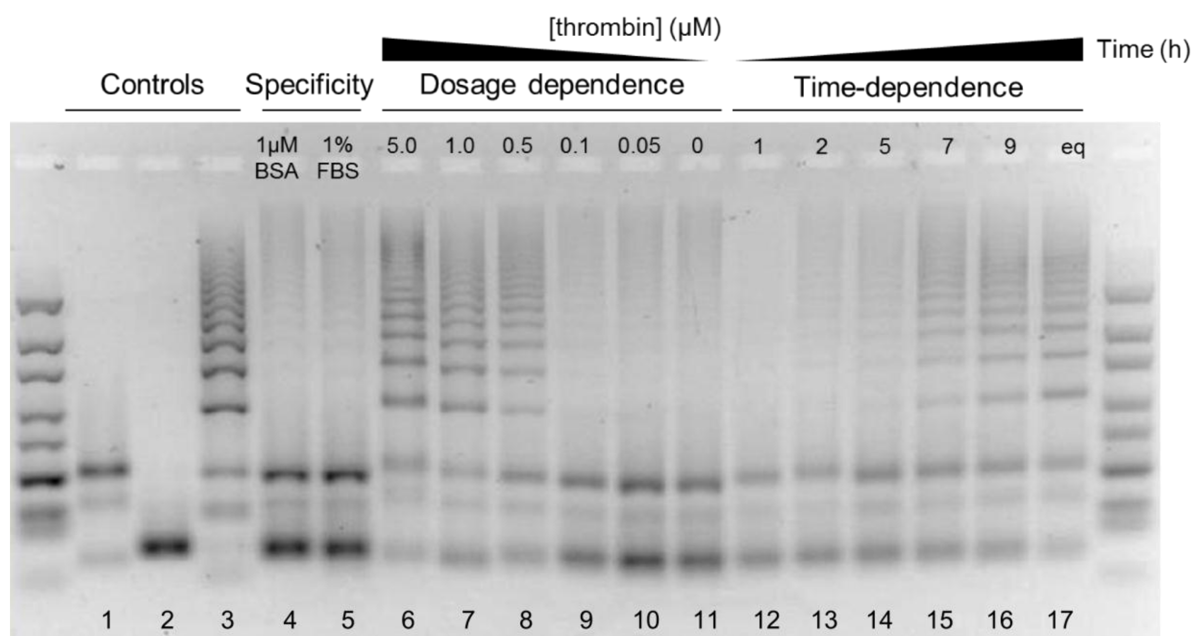

Figure S1. Gel electrophoresis image of the performance of proximity-based sensing DNA circuit evaluated at RT. Lane 1 corresponds to hairpins (HP1 and HP2) only; lane 2 corresponds to hairpins and initiator 2 – protector complex; lane 3 corresponds to that in lane 2 with an additional trigger strand. Hybridization chain reaction (HCR) was not triggered by non-specific protein (1 μM BSA, Lane 4) and interfering matrix (10% FBS, Lane 5). Lanes 6 – 11 represent the signal developed at different α-thrombin concentrations as labelled. Lanes 12 – 17 represent the kinetics of signal development with the equilibrium amount of HCR products attained only after ca. 7 h.

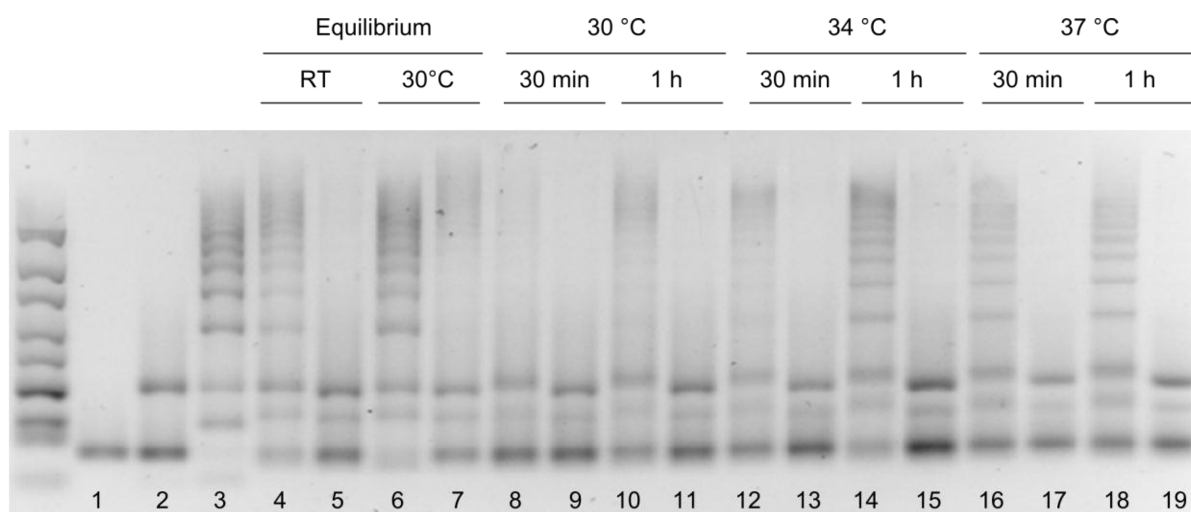

Figure S2. Gel electrophoresis image demonstrating the use of temperature to modulate the circuit kinetics. The temperature and time point of interest are labeled above the respective lanes. The left lane of each time point corresponds to the case where α-thrombin was added and the right lane corresponds to the absence of α-thrombin. As the temperature increased from 30 °C to 37 °C, more HCR products formed within 30 min, taking 1 h to be the reference lane for each temperature, which indicates a faster rate of reaction. The background leakage (HCR signal in absence of α-thrombin) was not significant within the analysis time of 1 h even at the highest temperature (37 °C) used. Thus, 37 °C was used in this study. The first three lanes are control lanes corresponding to: lane 1 – hairpin (HP1 and HP2) only; lane 2 – hairpin and initiator 2 – P complex; lane 3 – that in lane 2 with t* c* trigger strand for signal transduction.

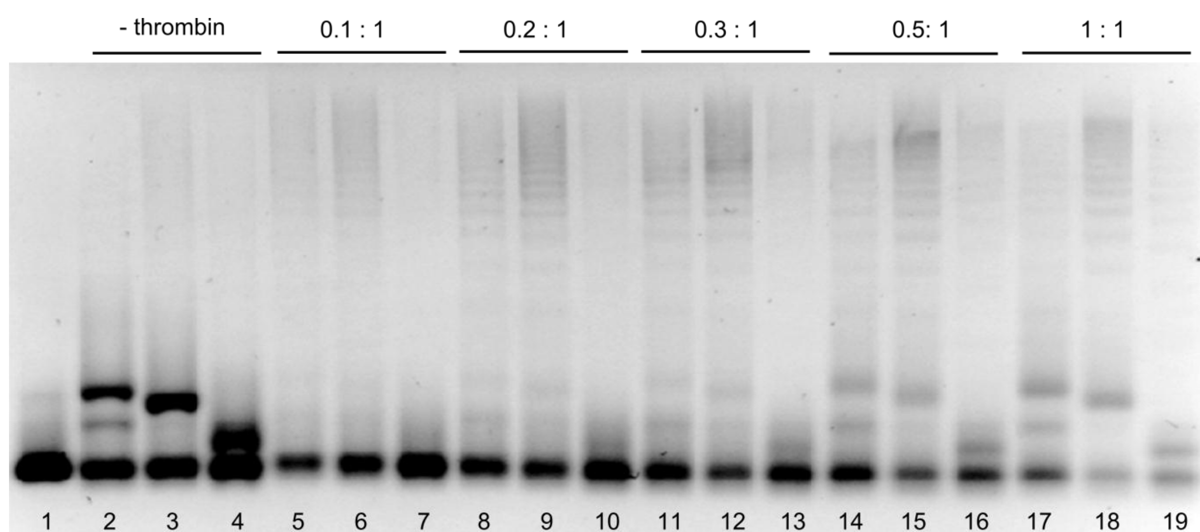

Figure S3. Gel electrophoresis image demonstrating the effect of the concentration of initiator 2 – P (I2-P) complex on the relative localized-to-non localized signal ratio. The respective ratios of I2-P complex to initiator 1 (I1) concentration are indicated above the relevant lanes. The I1 concentration was kept constant at 1 μM for all cases. The analysis time was kept constant at 15 min for all concentration ratios. For each region, the leftmost lane corresponds to the initiator of 15 nt spacer length (15T); center lane to that of 5 nt spacer length (5T); and rightmost lane to that without aptamer and spacer ("s") domain (noApt). Lane 1 corresponds to hairpins (HP1 and HP2) only; while lanes 2 – 4 correspond to the background signal in absence of α-thrombin. As the I2-P concentration increased, there was a general increase in the formation of HCR products. In all cases, the bound probes (15T and 5T) exhibited higher signal than the unbound noApt probe. The background noise from the unbound probes was more prominent at higher I2-P concentration. This highlights the need to keep the I2-P concentration low to minimize the development of non-localized signal. The I2-P to I1 ratio used for the subsequent part of the study was kept constant at 0.3.