Engineering transcription-based digital logic devices

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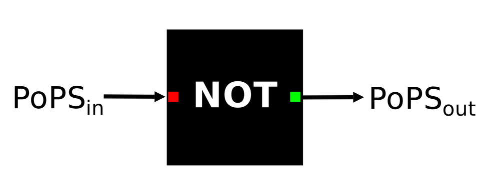
I Overview

Abstract

Implementing *in vivo* information processing is a key challenge in synthetic biology. We describe the construction and characterization of digital transcription-based devices from zinc fingers and leucine zippers. We also present a framework around device design and performance.

Goal

Implement *in vivo* combinational digital logic using transcription-based devices.



Desirable device features
1) composable
2) functional
3) scalable
4) well-characterized

Parts level implementation

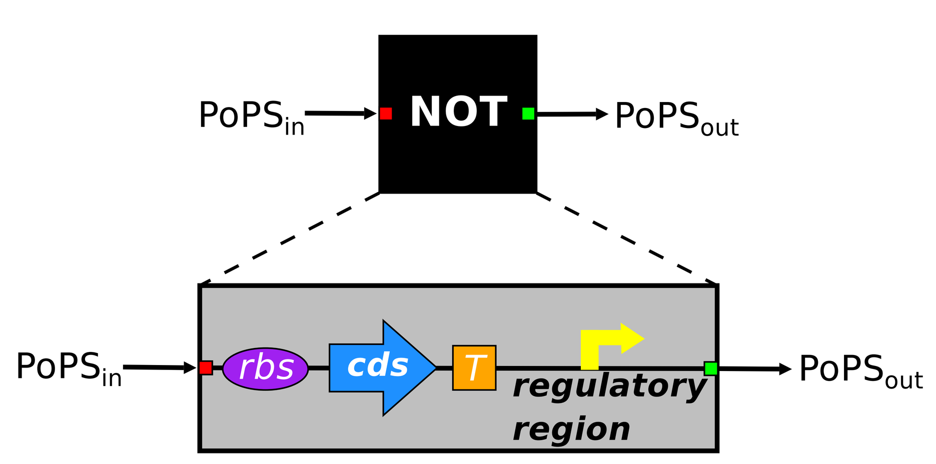


Figure 1: NOT devices can be implemented using 4 parts: an RBS (ribosome binding site), CDS (coding sequence), terminator and regulatory region.

Biological implementation

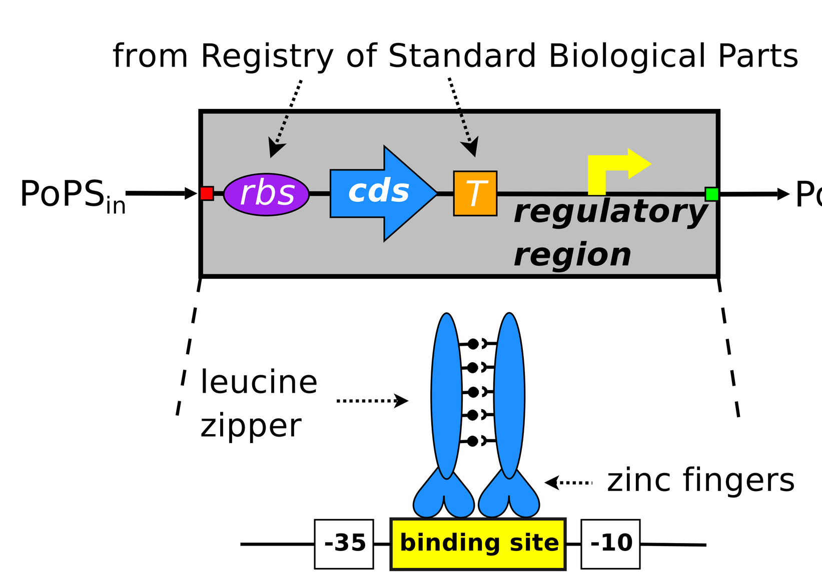


Figure 2: The CDS encodes a repressor that binds DNA (to repress transcription) and dimerizes (to exhibit cooperativity). The regulatory region binds repressor and has -35 and -10 sites that bind RNA polymerase to initiate transcription.

Device behavior

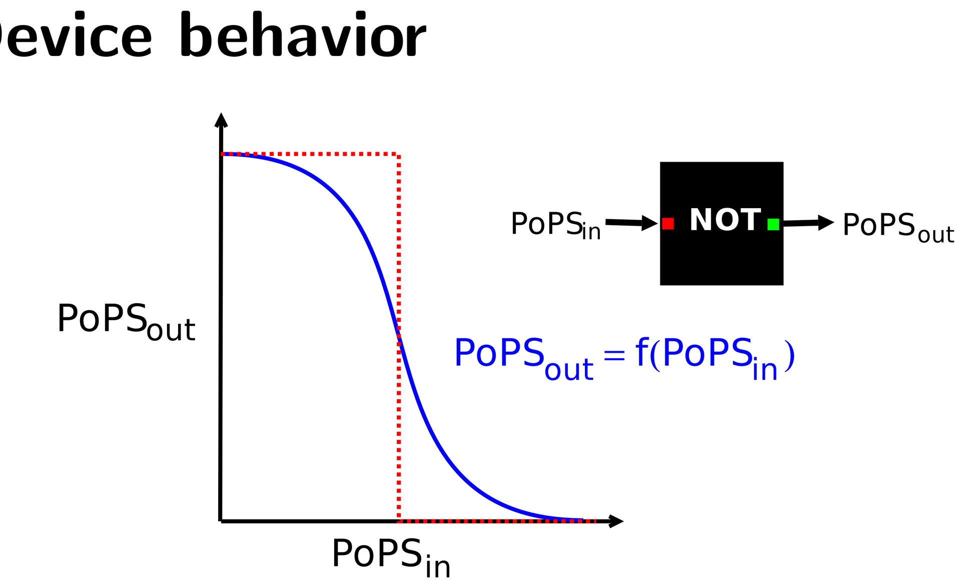


Figure 3: Static device function is described by a transfer characteristic: a plot of device output versus device input.

II Device performance framework

Performance metrics

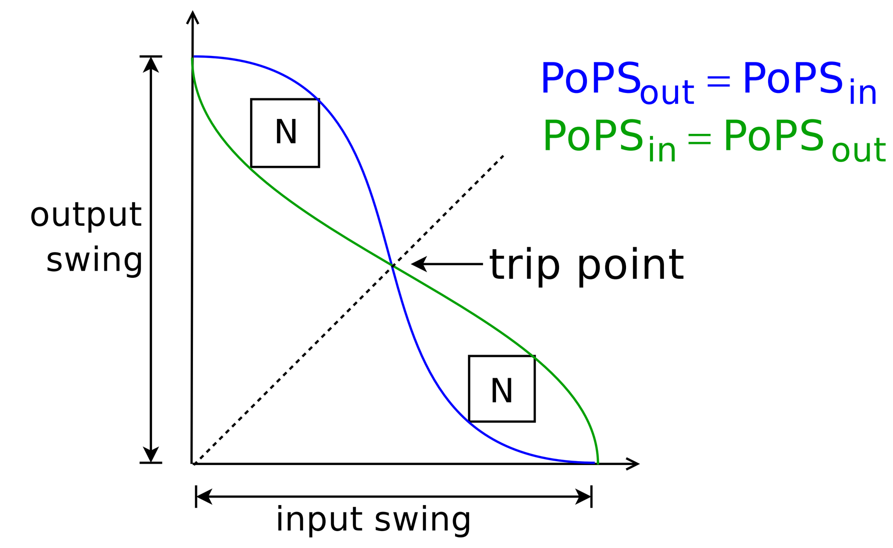


Figure 4: The swing, noise margin and trip point are the key metrics of device performance [1, 3]. Ideal devices maximize the noise margin and have a trip point close to half the device swing.

Question: What swing and noise margin do we need for reliable *in vivo* operation of transcription-based logic devices?

Noise in device signals lead to errors

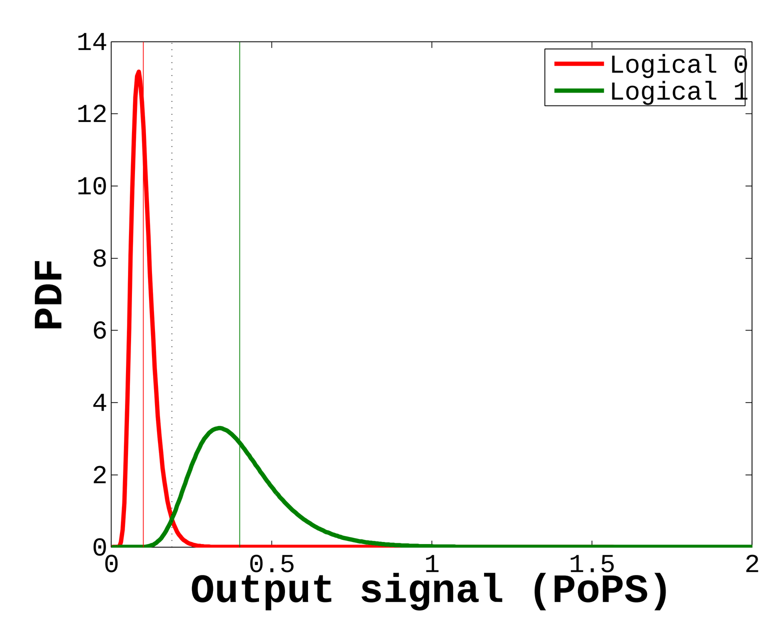


Figure 5: Device output signals are log-normally distributed [2]. Overlap in the signal distributions for logical 0 and logical 1 can lead to errors.

Error rate as a function of swing

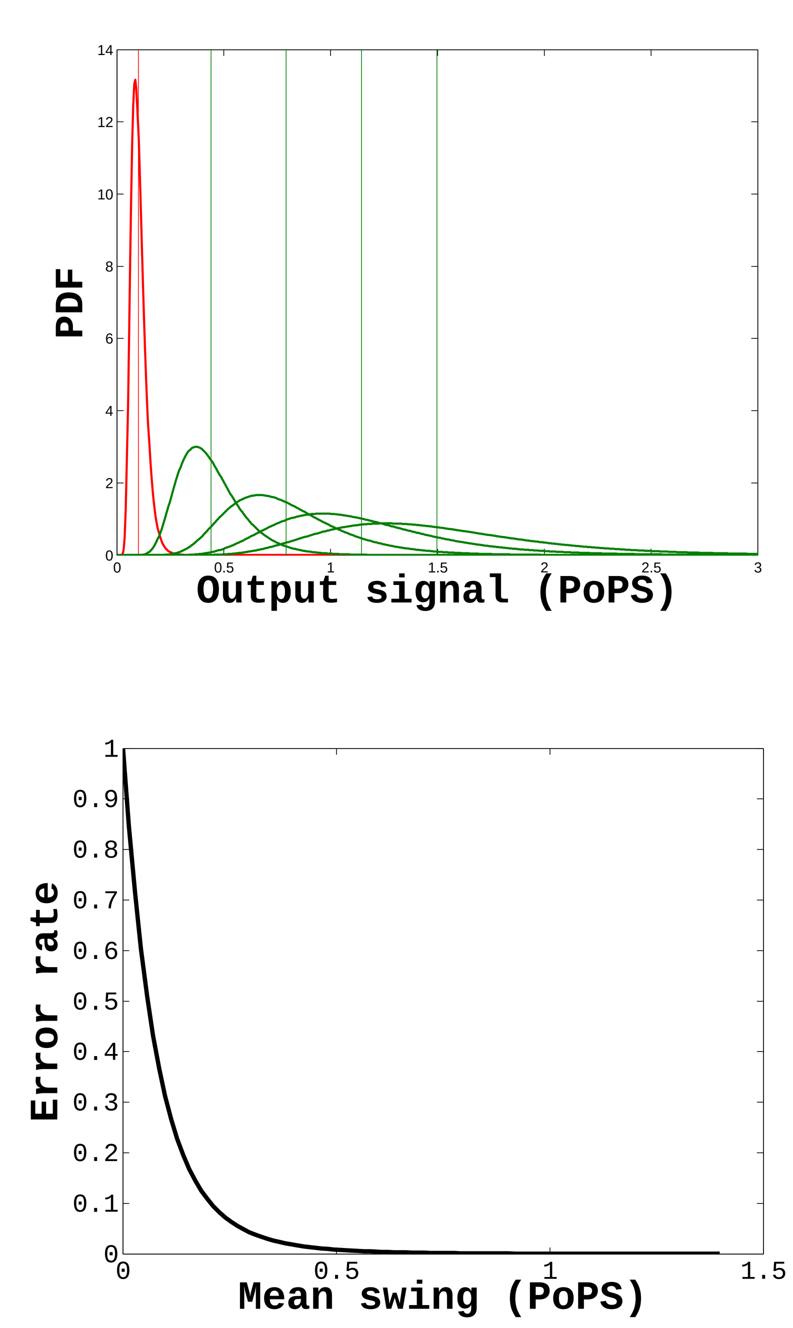


Figure 6: The device error rate decreases as swing increases.

Answer: The target device swing depends on the error rate we can tolerate in device operation. It is likely to vary according to the application. We need to choose an acceptable device error rate.

III Implementation

In vivo transfer curve

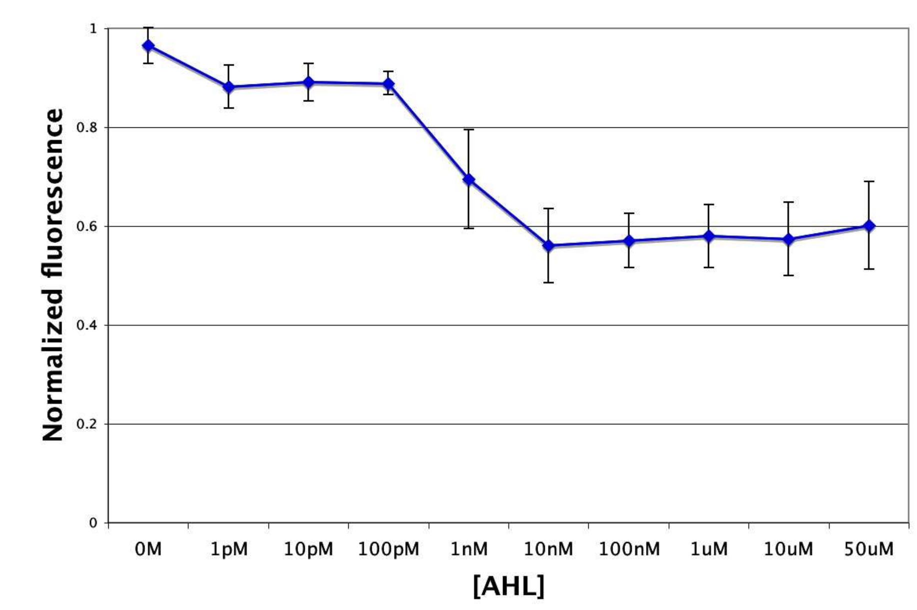


Figure 7: Normalized fluorescence versus inducer concentration for inverters BBa_Q20060.

In vitro transcriptional repression

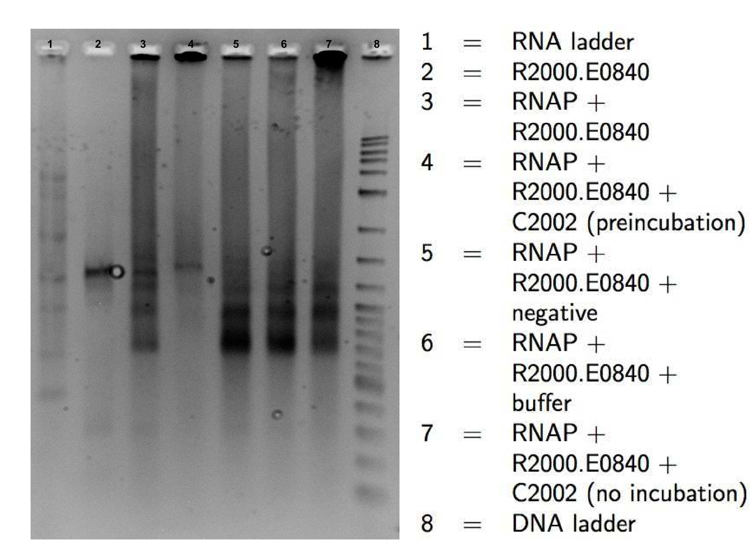


Figure 8: Preincubation of protein and regulatory region results in transcriptional repression.

Repressor expression is high

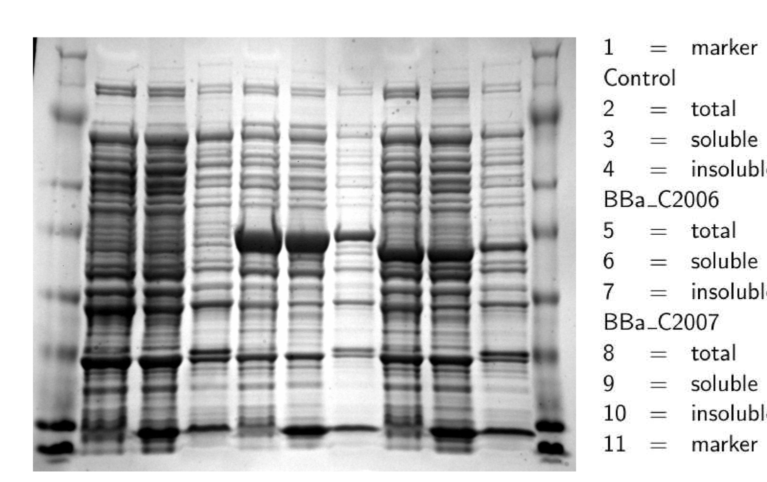


Figure 9: The repressor is expressed at high levels *in vivo*.

Future work

1. Improve repression *in vivo*.
2. Demonstrate scalability of design.

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