

# MATHEMATICS FOR BIOLOGICAL ENGINEERING: MODEL-DRIVEN DESIGN OF A SYNTHETIC BIO-LOGICAL AND GATE

Kavita Iyer Ramalingam, Jonathan Tomshine, Yiannis N. Kaznessis  
Department of Chemical Engineering & Materials Science, University of Minnesota  
421 Washington Ave SE, Minneapolis, MN 55455

Corresponding author e-mail: [yiannis@cems.umn.edu](mailto:yiannis@cems.umn.edu)

Using an integrated theoretical-experimental approach we engineered a synthetic novel transcriptional regulatory circuit with robust AND gate switch functionality in *E.coli*. The single promoter, hybrid system built from well characterized prokaryotic transcriptional building blocks (*lac* and *tet* operators, the promoter sequence of  $\lambda$ -phage) can be tuned with exogenous chemical moieties to exhibit the targeted phenotype. By manipulating operator positions we successfully generated AND gate phenotypes exhibiting varying induction thresholds from a single biological circuit. Interestingly, the placement of *lacO<sub>1</sub>* downstream of -10 hexamer in a multi-operator plasmid endowed the most stringent transcriptional control. Our detailed statistical thermodynamic and stochastic kinetic models can predict design feasibility, quantitatively explain the improved design and accurately capture leakiness of expression as a function of *lacO<sub>1</sub>* position. This study furnishes essential insight regarding effective *lacO<sub>1</sub>* position on the promoter thus facilitating high fidelity biological AND gates.

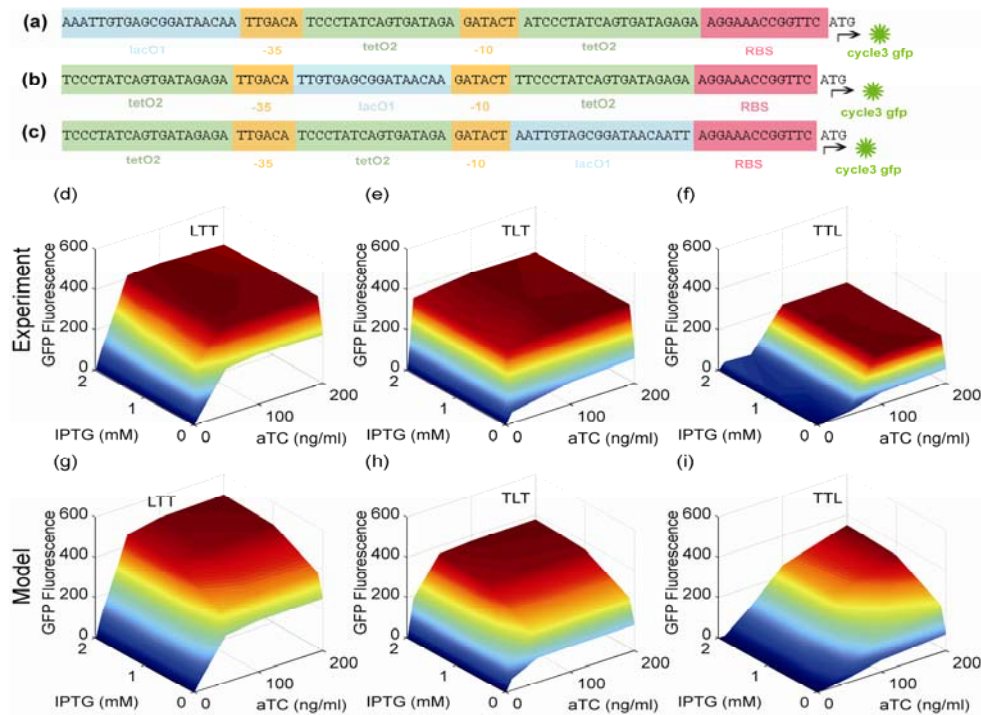
Numerous synthetic gene circuits (Hasty *et al*, 2002) have been created in the past decade, including bistable switches (Becskei and Serrano, 2000; Gardner *et al*, 2000; Hasty *et al*, 2002; Kramer *et al*, 2004), oscillators (Atkinson *et al*, 2003; Elowitz and Leibler, 2000), and logic gates (Guet *et al*, 2002; Kobayashi *et al*, 2004; Mayo *et al*, 2006; Setty *et al*, 2003). Most binary logical circuits built to date have wild type promoter systems, promoters with single-input regulator or multiple promoters governing genes encoding their corresponding transcriptional regulators in a network (Buchler *et al*, 2003; Guet *et al*, 2002; Mayo *et al*, 2006; Setty *et al*, 2003).

We devised an *in vivo* synthetic, hybrid system comprising of multiple operators on a single promoter, a novel, robust architecture from previously reported designs. The design strategy we envision as being simple, effective and new for generating synthetic AND logic gate relies on using a combination of three unrelated regulatory elements from natural systems of *tet*, *lac* and  $\lambda$ -phage operon arranged logically within a single transcriptional unit. Although use of different topology has resulted in post transcriptional logic functions (Rackham and Chin, 2005), we observe for the first time that engineering of promoter architecture by simple shuffling of just the operator positions enabled improved, high-fidelity AND gates.

Our system comprised of two *tet* and one *lac* operator sites in and around the P<sub>L</sub> ( $\lambda$ -phage) promoter permitted construction of three different regulatory motifs: TLT, TTL and LTT (Figure 1a-c), driving expression of green fluorescent protein (GFP). Thus the output fluorescence is dependant on input of two small molecule inducers, anhydrotetracycline (aTc) and iso-propyl- $\beta$ -thiogalactoside (IPTG). The implicit simplicity of these three designs is highlighted by the minimal number of regulatory components required to achieve high-fidelity, robust AND gate.

Following earlier *in silico* work (Tuttle *et al.*, 2005; Salis and Kaznessis, 2006; Salis *et al.*, 2006; Sotiropoulos and Kaznessis, 2007) we model all known biomolecular events involved in expression and regulation and we initially adopt a partition function based approach similar to that described by Arkin and coworkers (1998) and Shea and Ackers, (1985) to evaluate the feasibility of the proposed study. An equilibrium model incorporates all individual molecular species and interactions known to be involved in the synthetic transcriptional regulatory system, closely mimicking the endogenous environment. The probability of various potential promoter states is assessed, ultimately providing the probability of the system being in a transcriptionally-ready state,  $P_{init}$ . Total output fluorescence can then be modeled as  $\phi P_{init} + \chi$  where  $P_{init}$  accounts exclusively for the model's AND-gate behavior. Parameter  $\chi$  represents background fluorescence and  $\phi$  is an inducer-independent linear scaling factor dependent on the rates of transcription, translation, and degradation. Salient characteristics regarded as model strengths are that it accurately captures experimental data and provides a quantitative rationale for improved circuit behavior based on RNAP-repressor thermodynamics. The initial round of modeling based on literature thermodynamic parameters indicated that excellent AND gate behavior should be achievable with a synthetic hybrid *lac-tet* promoter. When the partition function describing the promoter is applied to a kinetic-stochastic model of transcription and translation, it faithfully portrays the experimentally observed distribution of GFP expression and the dynamic rise of fluorescence to a steady-state.

Figure 1 demonstrates that the integration of computational and experimental molecular biology can rationalize the synthesis of novel biological functionalities. We have constructed a high fidelity, bio-logical AND gate from a unique, simple, synthetic hybrid promoter module.



### Figure 1- Comprehensive overview of the synthetic hybrid bio-logical AND gates.

Architecture (LTT:1a, TLT:1b, TTL:1c), experimental outcome (LTT:1d, TLT:1e, TTL:1f) and corresponding model predictions (LTT:1g, TLT:1h, TTL:1i). 1a-c, promoter topology is varied by shifting *lacO* position from upstream of -35 to downstream of -10hexamer respectively. The *lacO* and *tetO* represent the binding sites for LacI and TetR. 1d-f, surface plots represent the mean of *gfp* fluorescence influenced by the grid of inducers concentrations (aTc, 0-200ng/ml; IPTG, 0-2mM); excellent agreement between the experimental results and model predictions. Presence of both inducers controlled *gfp* expression in a dose-dependant manner saturating at high concentrations. Notably induction thresholds in the presence of aTc and IPTG absent appeared to be a function of *lacO* location. The TTL promoter showed the best transcriptional regulation and AND gate phenotype of the 3 designs. The reduced GFP signal observed in the TTL operon's fully-induced states is possibly due to diminished promoter clearance by RNAP upon placement of *lacO* downstream of -10hexamer.

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