Mathematical Modeling of a Biphasic Isothermal DNA Amplification Reaction

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INTRODUCTION

Goal: Use biomarkers on miRNA to detect diseases and injuries such as malaria, tuberculosis (TB), and traumatic brain injuries (TBI's).

McroiRNA are very small, so amplification is necessary.

UDAR (Ultrasensitive DNA Amplification Reaction) [2]
- isothermal = inexpensive
- biphasic = potentially eliminates false positives

BACKGROUND

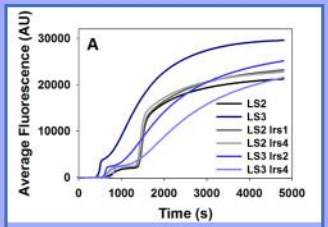

Figure 1: Black and gray lines represent Type I templates, and blue lines represent Type II templates.

Two types of looped templates that can be used in UDAR: Type I and Type II [2]
- Type I: steeper second rise giving higher potential to function as a digital on/off switch

Type II Model Assumptions [1]:
1. Multistep Binding
2. Polymerase Sequestration
3. Pseudo-Steady State
4. Constant Nickase Activity

METHODS

Due to the nonlinear relationship between concentration and fluorescence, current models are made to fit concentration data sets. Additionally, the pseudo-steady state assumption allows most parameters to be estimated by NUPACK [3]. The following reaction mechanisms are included in the most recent model:

- polymerase sequestration via Hill Equation with Multiple-Substrate Correction (C)
- exponential nickase deactivation

$$[\dot{Y}] = -2k_{on}[Y]^2 + 2k_{r_1}[YY] - k_{on}[Y][S] + k_{r_1}[YS] + k_y[N][W] - k_1[T][Y]^2$$

$$[\dot{YY}] = k_{on}[Y]^2 - k_{r_1}[YY] - k_p P_0 \frac{[YY]}{K_1 C}$$

$$[\dot{YS}] = k_{on}[Y][S] - k_{r_2}[YS] + k_p P_0 \frac{[YY]}{K_1 C} - k_p P_0 \frac{[YS]}{K_2 C}$$

$$[\dot{S}] = k_{r_2}[YS] - k_{on}[Y][S] - 2k_{on}[S]^2 + 2k_{r_3}[D]$$

$$[\dot{D}] = k_{on}[S]^2 - k_{r_3}[D] + k_p P_0 \frac{[YS]}{K_2 C}$$

$$[\dot{W}] = k_1[T][Y]^2 - k_y[N][W] + k_2 P_0 \frac{[V]}{K_3 C}$$

$$[\dot{V}] = k_y[N][W] - k_2 P_0 \frac{[V]}{K_3 C}$$

$$[\dot{N}] = -\beta[N]$$

$$C = 1 + \frac{[YY]}{K_1} + \frac{[YS]}{K_2} + \frac{[V]}{K_3}$$

$$[T] = T_0 - [W] - [V]$$

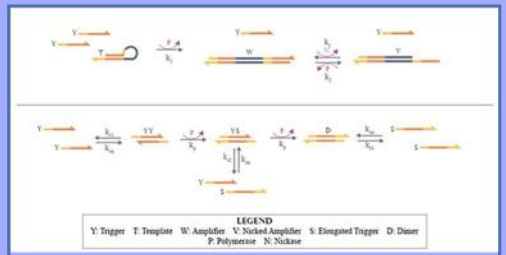

Figure 2: Current hypothesized reaction mechanisms. Curved arrows represent enzymatic activity.

RESULTS

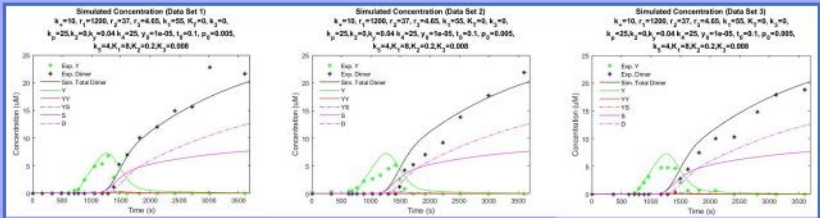

Figure 3: Output curve from MATLAB simulation of the above model over three LS2 data sets.

Despite the variations in each experimental data set, the model appears to mimic the behavior of all three data sets for the LS2 Type I template (Figure 3) . Modeling the concentrations of trigger and dimer is sufficient for modeling the entire reaction because all other reaction products stay at much lower concentrations.

CONCLUSIONS

- Nickase deactivation contributes to the plateau in dimer production at the end of the reaction.
- LS2 reactions are sufficiently modeled by the current model.
- Steep second rise in Type I might be artificial due to a difference in fluorescence.
  - This hypothesis is backed by recent data collected in Dr. McCalla's lab.

FUTURE WORK

- Apply model to other templates.
- Use second-phase data to fit K values.
- Try several different fluorescence conversions to untangle the relationship between concentration and fluorescence output.
- Create equations to link Type II and Type I models to create a universal model
  - Create equation to predict K values using thermodynamic values from NUPACK
  - Analyze difference in concentration - fluorescence relationship for each template

ACKNOWLEDGEMENTS

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103474. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Other funding came from an NSF REU under award supplement 1847245 for Dr. Stephanie McCalla.

REFERENCES

1. Ciesielski, D., Özay, B., McCalla, S. and Gedeon, T. A mathematical model for a biphasic DNA amplification reaction, Interface Royal Society, Volume 16, Issue 154 (2019),
2. Özay, B., Robertus, C. M., Negri, J. L., and McCalla, S. E. (2018) Biphasic, Switch-Like Isothermal DNA Amplification. Analyst, 2018,143, 1820-1828
3. Zadeh, J. N., Steenberg, C.D., Bois, L.S., Wolfe, B.R., Pierce, M. B., Khan, A.R., Dirks, R.M., Pierce, N.A. NUPACK: analysis and design of nucleic acid systems. J Comput Chem, 32:170–173, 2011. (pdf)