

# Modelling RNA Circuits

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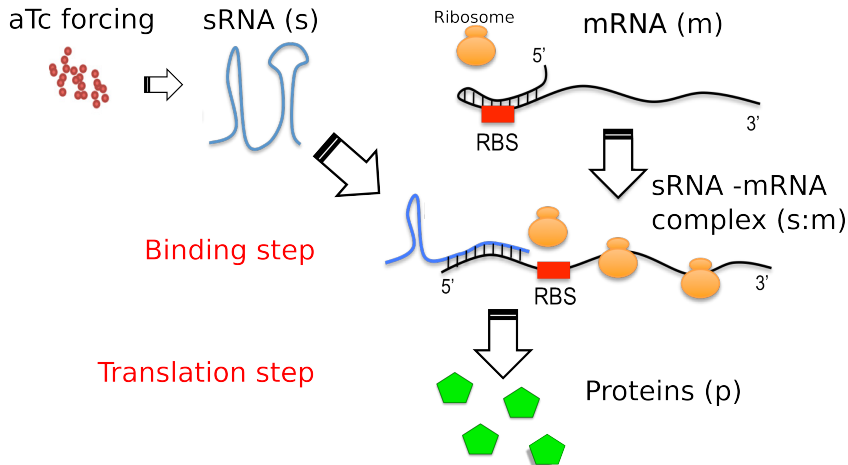
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# Objectives

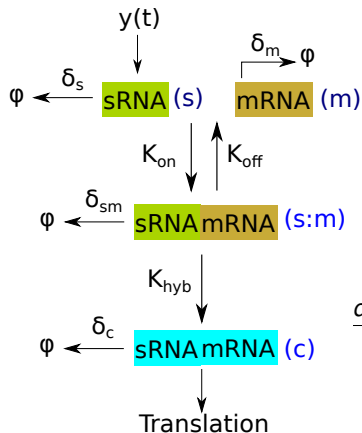
- ▶ Goal in synthetic biology - manipulate natural cellular machinery
- ▶ Rational design of genetic circuits now possible
  - ▶ ODE models used for prediction.
  - ▶ Model parameters unknown.
- ▶ Project studied a recently designed genetic circuit
- ▶ **Aim:** use **ODE model** with **recent data** to perform **parameter estimation**.
- ▶ Parameter estimates would inform future design/experiments.

# The RNA System



- ▶ mRNA produced 'self repressed' - tail folded over the RBS
- ▶ sRNA binds to it - new complex has RBS uncovered.
- ▶ Complex translated into proteins.

# ODE model for system (1) - Binding step



- Binding of sRNA ( $s$ ) and mRNA ( $m$ ), into unstable complex ( $s : m$ ) then stable one ( $c$ ).

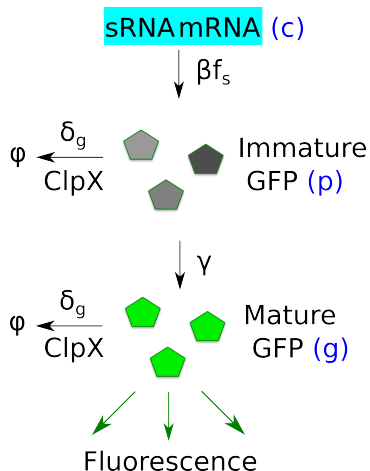
$$\frac{ds}{dt} = \frac{N_{\alpha_T}}{f_T} y(t) - (\mu + \delta_s)s - k_{on}sm + k_{off}s : m$$

$$\frac{dm}{dt} = \frac{N_{\alpha_L}}{f_L} x(t) - (\mu + \delta_m)m - k_{on}sm + k_{off}s : m$$

$$\frac{ds : m}{dt} = k_{on}sm - (k_{off} + k_{hyb})s : m - (\mu + \delta_{sm})s : m$$

$$\frac{dc}{dt} = k_{hyb}s : m - (\mu + \delta_c)c$$

# ODE model for system (2) - Translation step



- ▶ Translation of stable complex (c) into immature GFP (p).
- ▶ maturation of GFP (g), machine calibration giving measured fluorescence (z).

$$\frac{dp}{dt} = \beta m + f_s \beta c - (\gamma + \mu + \delta_g)p - \frac{v_z p}{K_z + p + g}$$

$$\frac{dg}{dt} = \gamma p - (\mu + \delta_g)g - \frac{v_z g}{K_z + p + g}$$

$$z = z_0 + \frac{g}{\Theta}$$

# Full model, with parameters to be estimated

$$\frac{ds}{dt} = \frac{N_{\alpha_T}}{f_T} y(t) - (\mu + \delta_s)s - k_{\text{on}}sm + k_{\text{off}}s : m \quad (1)$$

$$\frac{dm}{dt} = \frac{N_{\alpha_L}}{f_L} x(t) - (\mu + \delta_m)m - k_{\text{on}}sm + k_{\text{off}}s : m \quad (2)$$

$$\frac{ds : m}{dt} = k_{\text{on}}sm - (k_{\text{off}} + k_{\text{hyb}})s : m - (\mu + \delta_m)s : m \quad (3)$$

$$\frac{dc}{dt} = k_{\text{hyb}}s : m - (\mu + \delta_m)c \quad (4)$$

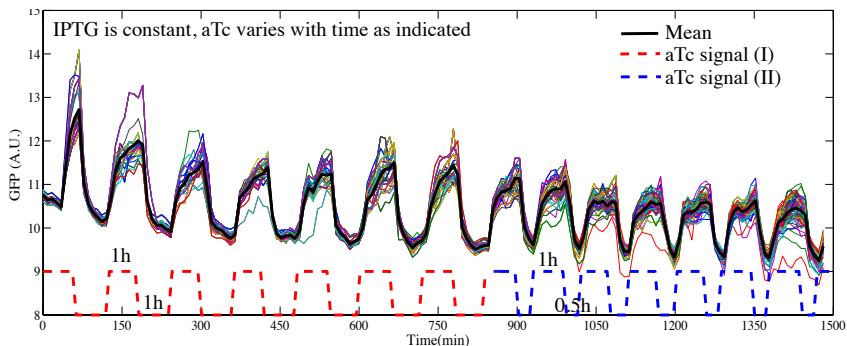
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$$\frac{dp}{dt} = \beta m + f_s \beta c - (\gamma + \mu + \delta_g)p - \frac{v_z p}{K_z + p + g} \quad (5)$$

$$\frac{dg}{dt} = \gamma p - (\mu + \delta_g)g - \frac{v_z g}{K_z + p + g} \quad (6)$$

$$z = z_0 + \frac{g}{\ominus} \quad (7)$$

# Recent experimental data



- ▶ Data records  $z = z_0 + \frac{g}{\Theta}$
- ▶ Single cell fluorescence time series data (Jaramillo Lab, unpublished).

# Method for parameter estimation in ODE model

## Goal

Estimate unknown parameters in model using time series data.

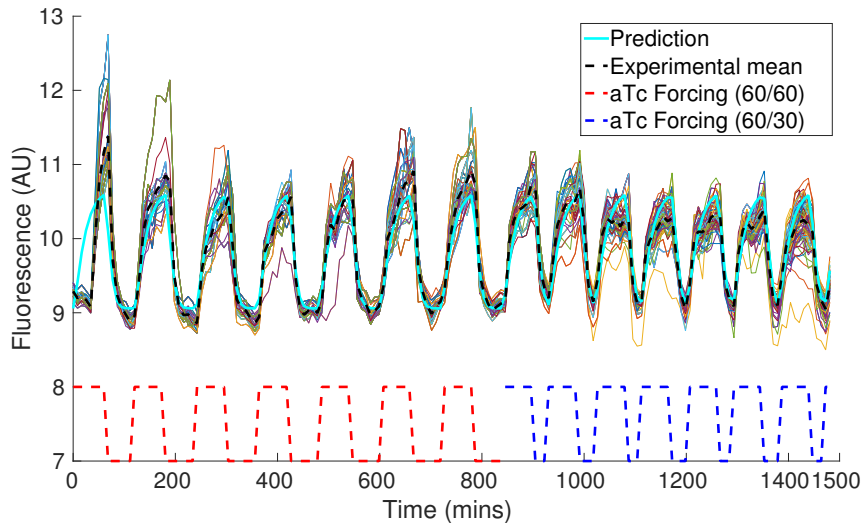
- ▶ Least squares error minimization approach

$$\arg \min_{\theta} \sum_{i=1}^n (z_{\text{exp,mean}}(t_i) - z(t_i, \theta))^2.$$

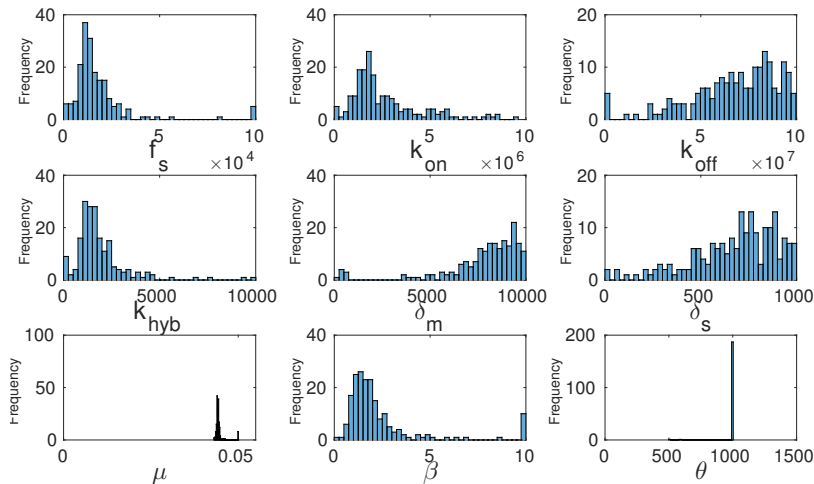
- ▶ Used evolutionary algorithm to perform minimisation.



# Initial estimation results



# Initial estimation results

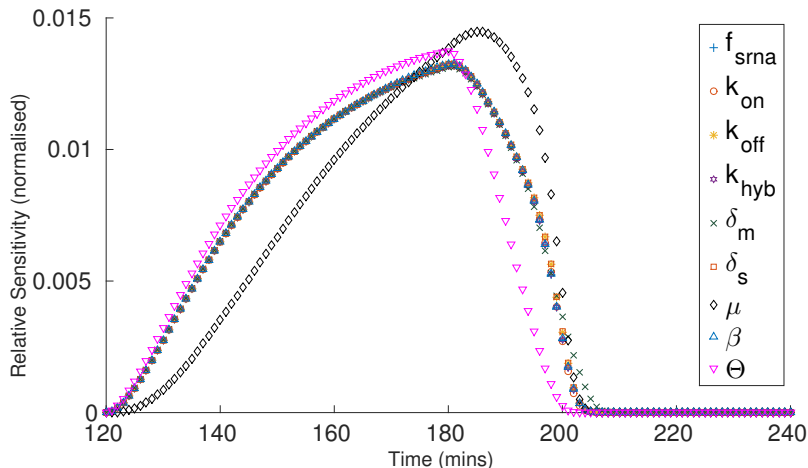


- ▶ Many parameters poorly estimated.
- ▶ Similar error values for all results.

# Sensitivity analysis

$$S_{ij} = \left. \frac{\partial z}{\partial \theta_j} \right|_{t_i}$$

Perturbations of parameters all give similar effects on model output - **hard to distinguish**



# Plotting all states of model output

$$\frac{ds}{dt} = \frac{N_{\alpha_T}}{f_T} y(t) - (\mu + \delta_s)s - k_{\text{on}}sm + k_{\text{off}}s : m$$

$$\frac{dm}{dt} = \frac{N_{\alpha_L}}{f_L} x(t) - (\mu + \delta_m)m - k_{\text{on}}sm + k_{\text{off}}s : m$$

$$\frac{ds : m}{dt} = k_{\text{on}}sm - (k_{\text{off}} + k_{\text{hyb}})s : m - (\mu + \delta_m)s : m$$

$$\frac{dc}{dt} = k_{\text{hyb}}s : m - (\mu + \delta_m)c$$

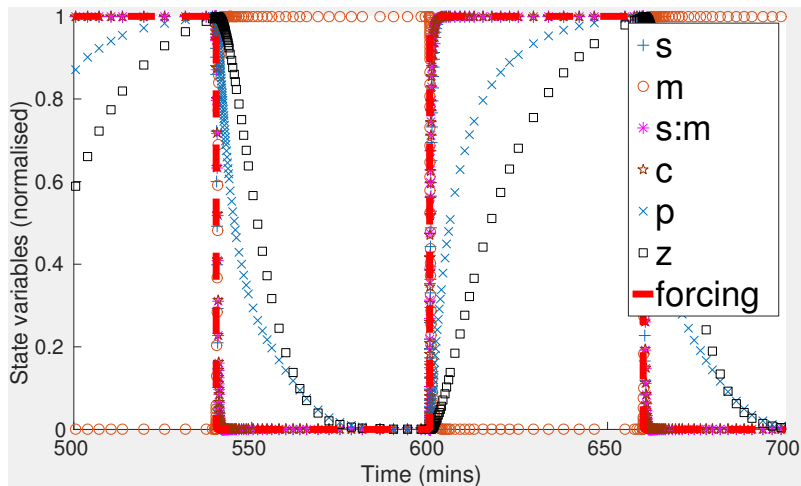
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$$\frac{dp}{dt} = \beta m + f_s \beta c - (\gamma + \mu + \delta_g)p - \frac{v_z p}{K_z + p + g}$$

$$\frac{dg}{dt} = \gamma p - (\mu + \delta_g)g - \frac{v_z g}{K_z + p + g}$$

$$z = z_0 + \frac{g}{\ominus}$$

# Model output for all state variables



- ▶  $s, m, s : m, c$  (binding step) respond instantly to forcing.
- ▶  $p, z$  (translation step) on timescale of forcing.

# Translation rate limiting

$$\begin{aligned}\frac{ds}{dt} &= \frac{N\alpha_T}{f_T} y(t) - (\mu + \delta_s)s - k_{\text{on}}sm + k_{\text{off}}s : m \\ \frac{dm}{dt} &= \frac{N\alpha_L}{f_L} x(t) - (\mu + \delta_m)m - k_{\text{on}}sm + k_{\text{off}}s : m \\ \frac{ds : m}{dt} &= k_{\text{on}}sm - (k_{\text{off}} + k_{\text{hyb}})s : m - (\mu + \delta_m)s : m \\ \frac{dc}{dt} &= k_{\text{hyb}}s : m - (\mu + \delta_m)c\end{aligned}$$


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$$\begin{aligned}\frac{dp}{dt} &= \beta m + f_s \beta c - (\gamma + \mu + \delta_g)p - \frac{v_z p}{K_z + p + g} \\ \frac{dg}{dt} &= \gamma p - (\mu + \delta_g)g - \frac{v_z g}{K_z + p + g} \\ z &= z_0 + \frac{g}{\Theta}\end{aligned}$$

- ▶ Binding equations respond instantly.
- ▶  $\beta m + f_s \beta c$  term links binding and translation. It flips between fixed point values.
- ▶ All parameters 'upstream' have identical effects.

# Simplified model

$$\frac{ds}{dt} = \frac{N\alpha_T}{f_T} y(t) - (\mu + \delta_s)s - k_{\text{on}}sm + k_{\text{off}}s : m$$

$$\frac{dm}{dt} = \frac{N\alpha_L}{f_L} x(t) - (\mu + \delta_m)m - k_{\text{on}}sm + k_{\text{off}}s : m$$

$$\frac{ds : m}{dt} = k_{\text{on}}sm - (k_{\text{off}} + k_{\text{hyb}})s : m - (\mu + \delta_{sm})s : m$$

$$\frac{dc}{dt} = k_{\text{hyb}}s : m - (\mu + \delta_c)c$$

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$$\frac{dp}{dt} = Fy(t) - (\gamma + \mu + \delta_g)p - \frac{v_z p}{K_z + p + g}$$

$$\frac{dg}{dt} = \gamma p - (\mu + \delta_g)g - \frac{v_z g}{K_z + p + g}$$

$$z = z_0 + \frac{g}{\ominus}$$

# Simplified model

$$\frac{dp}{dt} = Fy(t) - (\gamma + \mu + \delta_g)p - \frac{v_z p}{K_z + p + g} \quad (8)$$

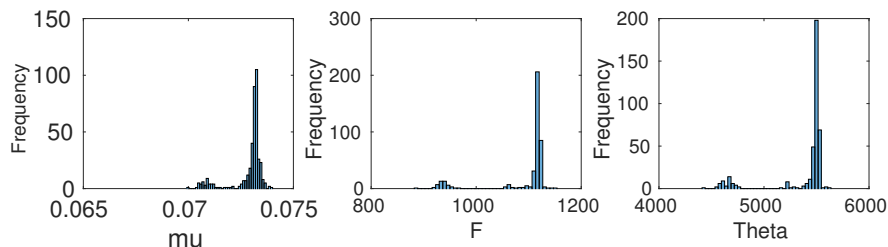
$$\frac{dg}{dt} = \gamma p - (\mu + \delta_g)g - \frac{v_z g}{K_z + p + g} \quad (9)$$

$$z = z_0 + \frac{g}{\Theta} \quad (10)$$

- ▶ Model only rate limiting steps - force translation term directly.
- ▶ 3 unknown parameters

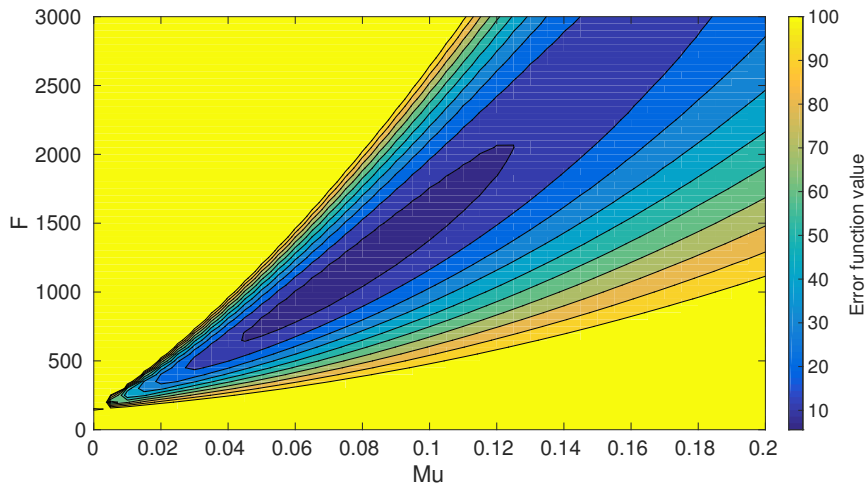


# Simplified model results (1)



- ▶ Error values as low as full model
- ▶ Clearer parameter estimation results
- ▶ Two peak structure due to local minima

## Simplified model results (2)



- Error landscape,  $\Theta = 5400$ .

# Conclusions and further work

- ▶ Fluorescence data **not enough** to estimate all unknown parameters
  - ▶ Simplified model not helpful in estimating original parameters.

To get these parameters ...

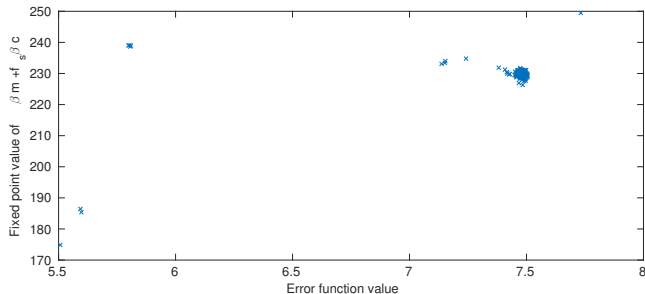
- ▶ Further experiments needed - observe fast timescale.
- ▶ Improve methodology
  - ▶ Algorithms for dealing with complex chemical models & limited data.
  - ▶ Bayesian Methods

# Acknowledgements

- ▶ Manish Kushawaha, Jaramillo Lab - School of Life Sciences
- ▶ Shenshi Shen, Boris Kisov for data.
- ▶ Annabelle Ballesta - Systems Biology

# Additional Slides

# Model fixed point for estimated parameters



- ▶ Similar fixed point values of translation forcing term,  $\beta m + f_s \beta c$ , across estimated parameter sets.
- ▶ Suggests translation may be rate limiting step.