Modelling RNA Circuits

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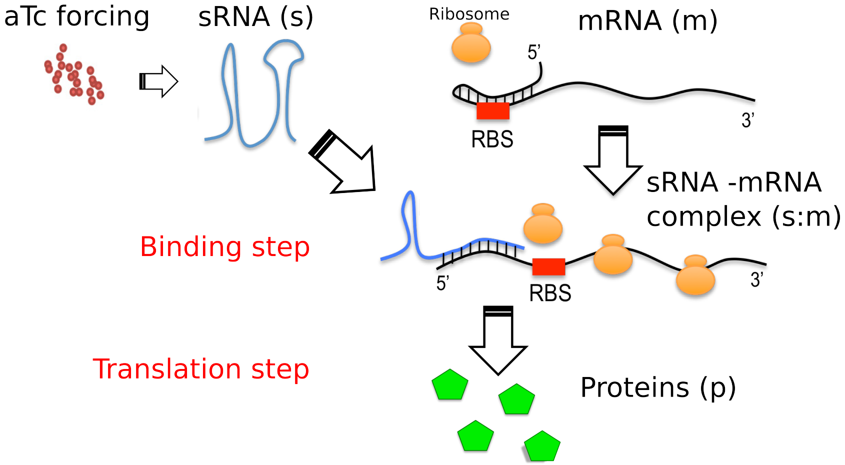
Objectives

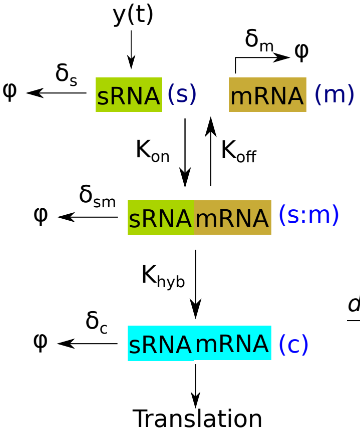
- Rational design of genetic circuits now possible
  - ODE models used for prediction.
  - Model parameters unknown.
- Project studied a recently designed genetic circuit, attempted parameter estimation.
- Parameter estimates would inform future design/experiments.

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).

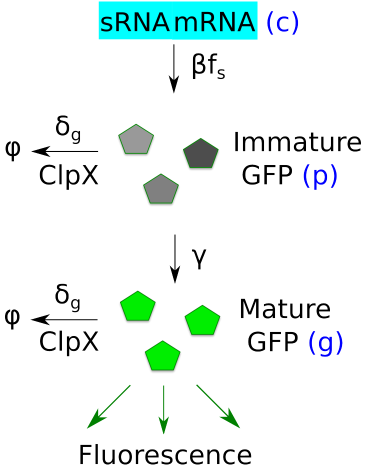
$$\frac{ds}{dt} = \frac{N\alpha_T}{f_T} y(t) - (\mu + \delta_s)s - k_{\text{on}} sm + k_{\text{off}} s:m$$

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

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

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

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

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

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

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

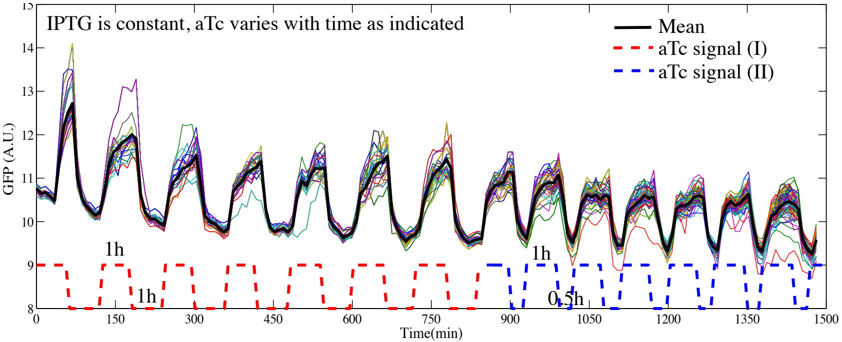
$$\frac{dc}{dt} = k_{\mathrm{hyb}} s:m - (\mu + \delta_m)c \tag{4}$$

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

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

$$z = z_0 + \frac{g}{\Theta} \tag{7}$$

Recent experimental data



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- Single cell fluoresence time series data (Jaramillo Lab, unpublished).

Method for parameter estimation in ODE model

Goal

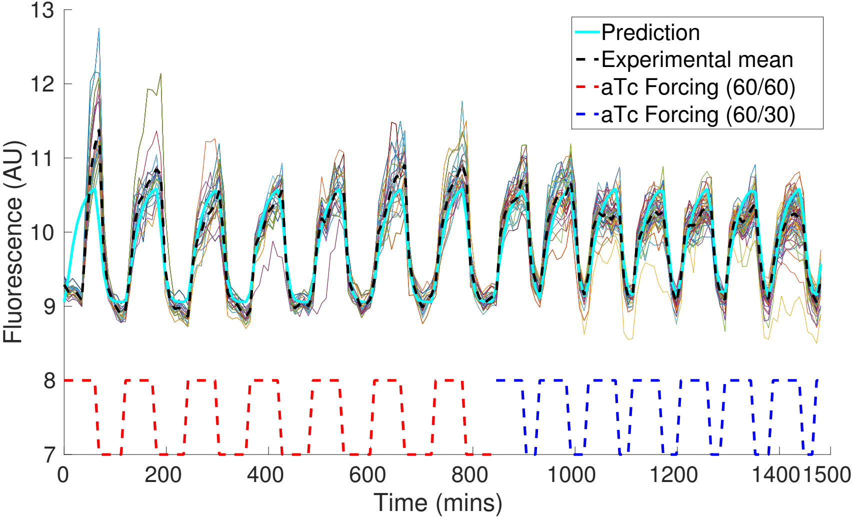
Estimate unknown parameters in model using time series data.

- Least squares error minimization approach

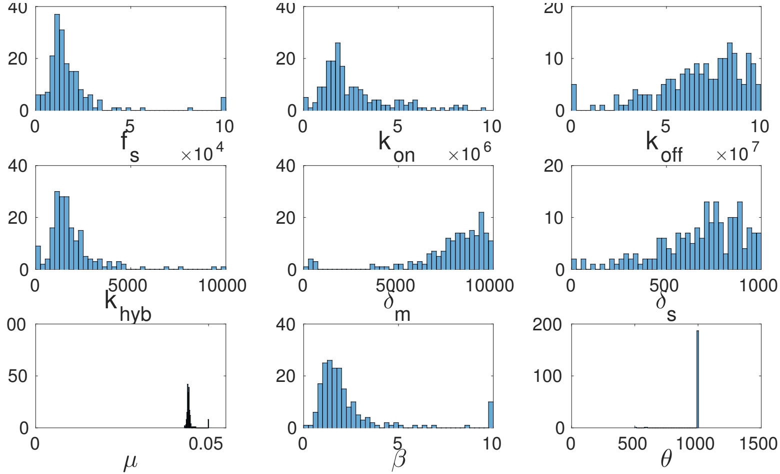
$$\arg\min_{\boldsymbol{\theta}} \sum_{i=1}^{n} (z_{\text{exp,mean}}(t_i) - z(t_i, \boldsymbol{\theta}))^2.$$

- Used evolutionary algorithm to perform minimisation.

Initial estimation results



Initial estimation results

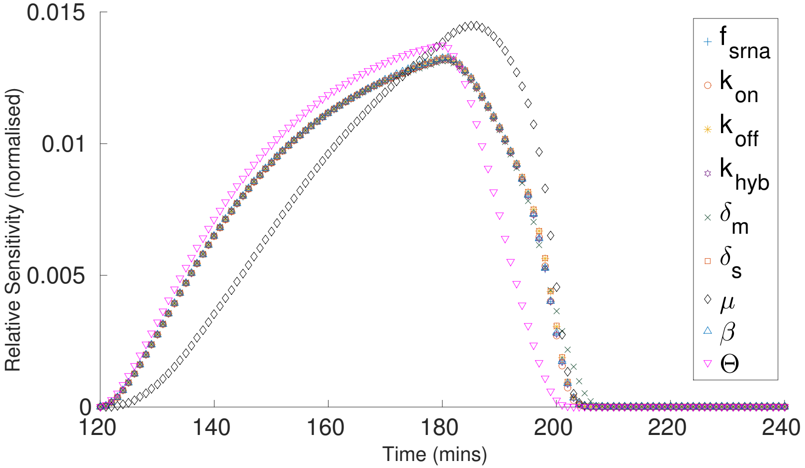


- Many parameters poorly estimated.
- Similar error values for all results.

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

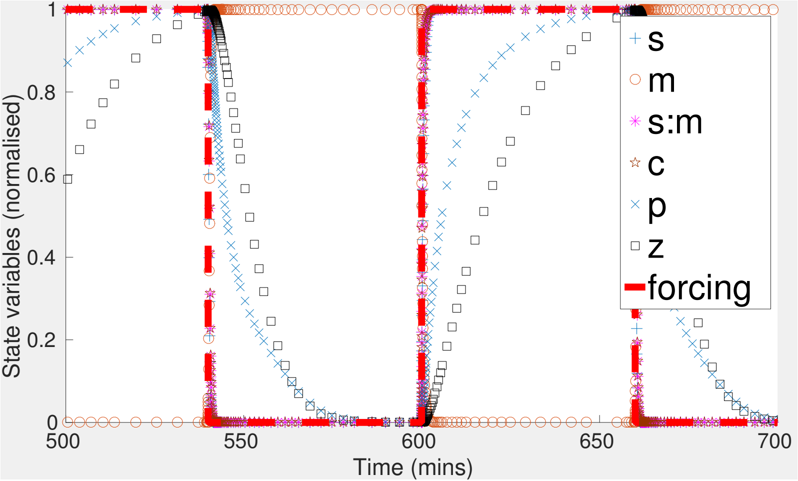


Plotting all states of model output

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

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

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

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

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

- 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

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

$$
\begin{aligned}
\frac{dp}{dt} &= F y(t) - (\gamma + \mu + \delta_g)p - \frac{v_z p}{K_z + p + g} \\
\frac{dg}{dt} &= \gamma p - (\mu + \delta_g)g - \frac{v_z g}{K_z + p + g} \\
z &= z_0 + \frac{g}{\Theta}
\end{aligned}
$$

Simplified model

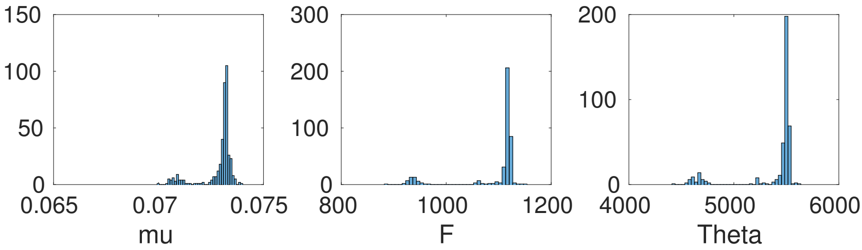
$$\frac{dp}{dt} = Fy(t) - (\gamma + \mu + \delta_g)p - \frac{v_z p}{K_z + p + g} \tag{8}$$

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

$$z = z_0 + \frac{g}{\Theta} \tag{10}$$

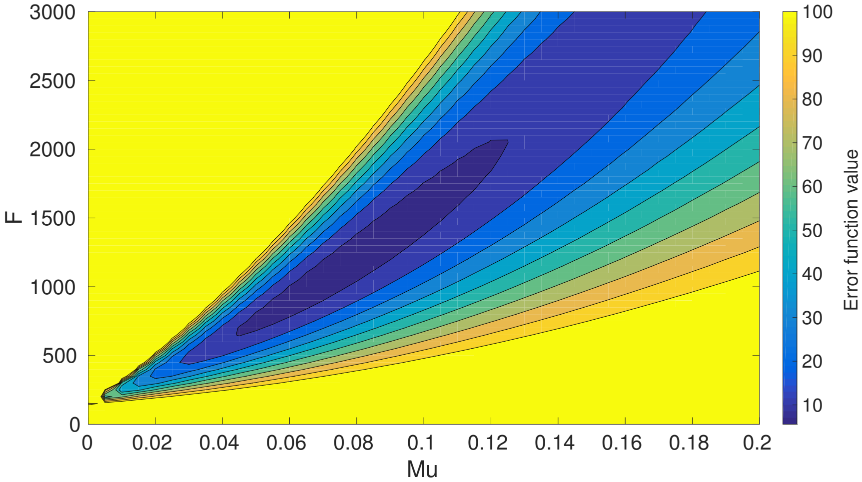
- Model only rate limiting steps - force translation term directly.
- 3 unknown parameters

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, $\Theta = 5400$.

Future work

- Fluorescence data not enough to estimate all unknown parameters
- Further experiments needed - observe fast timescale.
- Investigate methods of McAuley et al.
- Change methodology - Bayesian Methods?

Acknowledgements

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- Additional Slides