A second study describes the development of a genetic oscillator based on the combination of positive and negative feedback:

M. R. Atkinson, M. A. Savageau, J. T. Myers, and A. J. Ninfa. *Cell* **113**, 597-607 (2003)

In this lecture we will derive the stability diagram in Fig. 1B. In the model odd subscripts are used for mRNA whereas even subscripts are used for proteins. For example, the translation of mRNA is modeled as:

$$\frac{dX_2}{dt} = k_p X_1 - \beta_2 X_2 \qquad \textbf{[VII.17]}$$

where k_p is the translation rate constant and β_2 is the decay rate constant of the protein X_2. When X_2 and X_1 are normalized to their steady state values: $X_2^{ss} = \frac{k_p}{\beta_2} X_1^{ss}$, [VII.7] takes the form:

$$\frac{dx_2}{dt} = \beta_2 (x_1 - x_2) \qquad \textbf{[VII.18]}$$

Analogously the system of equations describing the genetic circuit in Fig.1A is:

$$\begin{aligned}
\frac{dx_1}{dt} &= \beta_1 (f_1 - x_1) \\
\frac{dx_2}{dt} &= \beta_2 (x_1 - x_2) \\
\frac{dx_3}{dt} &= \beta_3 (f_3 - x_3) \\
\frac{dx_4}{dt} &= \beta_4 (x_3 - x_4) \\
\frac{dx_5}{dt} &= \beta_5 (f_5 - x_5) \\
\frac{dx_6}{dt} &= \beta_6 (x_5 - x_6)
\end{aligned} \qquad \textbf{[VII.19]}$$

The functions f_1, f_3, and f_5 describe the transcriptional regulation and are defined by tri-phasic functions. For the stability analysis only the first four equations are relevant since no feedback occurs after x_4. As described in the Supplementary information of the paper:

$$f_1 = \begin{cases} B & : \quad x_2^{g_{12}} x_4^{g_{14}} < B \\ x_2^{g_{12}} x_4^{g_{14}} & : B < x_2^{g_{12}} x_4^{g_{14}} < M \\ M & : \quad x_2^{g_{12}} x_4^{g_{14}} > M \end{cases} \qquad \textbf{[VII.20a]}$$

$$f_3 = \begin{cases} B & : \quad x_2^{g_{32}} < B \\ x_2^{g_{32}} & : B < x_2^{g_{32}} < M \\ M & : \quad x_2^{g_{32}} > M \end{cases} \qquad \textbf{[VII.20b]}$$

In the case of a single fixed point, this point occurs at $x_1=x_2=x_3=x_4=1$. The matrix A is now defined as:

$$A = \begin{bmatrix} -\beta_1 & \beta_1 g_{12} & 0 & \beta_1 g_{14} \\ \beta_2 & -\beta_2 & 0 & 0 \\ 0 & \beta_3 g_{32} & -\beta_3 & 0 \\ 0 & 0 & \beta_4 & -\beta_4 \end{bmatrix} \qquad \textbf{[VII.21]}$$

The eigenvalues of this matrix are found by solving:

$$\begin{vmatrix} -\beta_1 - \lambda & \beta_1 g_{12} & 0 & \beta_1 g_{14} \\ \beta_2 & -\beta_2 - \lambda & 0 & 0 \\ 0 & \beta_3 g_{32} & -\beta_3 - \lambda & 0 \\ 0 & 0 & \beta_4 & -\beta_4 - \lambda \end{vmatrix} = 0 \qquad \textbf{[VII.22]}$$

This leads to the characteristic equation in the form:

$$\begin{aligned} & a_o \lambda^4 + a_1 \lambda^3 + a_2 \lambda^2 + a_3 \lambda + a_4 = 0 \\ & a_o = 1 \\ & a_1 = \beta_1 + \beta_2 + \beta_3 + \beta_4 \\ & a_2 = \beta_1 \beta_2 (1 - g_{12}) + \beta_1 \beta_3 + \beta_1 \beta_4 + \beta_2 \beta_3 + \beta_2 \beta_4 + \beta_3 \beta_4 \\ & a_3 = \beta_1 \beta_2 \beta_3 (1 - g_{12}) + \beta_1 \beta_2 \beta_4 (1 - g_{12}) + \beta_2 \beta_3 \beta_4 + \beta_1 \beta_3 \beta_4 \\ & a_4 = \beta_1 \beta_2 \beta_3 \beta_4 (1 - g_{14} g_{32} - g_{12}) \end{aligned} \qquad \textbf{[VII.23]}$$

Solving for the λ's is difficult. However there is a convenient mathematical condition, called the Routh-Hurwitz criterion that allows you to determine the stability without explicitly calculating the eigenvalues. The Routh-Hurwitz criterion states that a system is stable (real part of all eigenvalues is negative) if all coefficients [VII.23] are positive and all elements in the first column of the Routh-Hurwitz matrix are positive. This matrix is constructed as follows:

The matrix has n+1 (in our case 5) rows:

$$\begin{array}{c|ccccc}
\lambda^n & a_o & a_2 & a_4 & a_6 & \cdots \\
\lambda^{n-1} & a_1 & a_3 & a_5 & a_7 & \cdots \\
\lambda^{n-2} & b_1 & b_2 & b_3 & b_4 & \cdots \\
\lambda^{n-3} & c_1 & c_2 & c_3 & c_4 & \cdots \\
\lambda^{n-4} & d_1 & d_2 & d_3 & d_4 & \cdots \\
\vdots & \vdots & \vdots & \vdots & \vdots & \\
\lambda^1 & f_1 & & & & \\
\lambda^0 & g_1 & & & &
\end{array} \qquad \textbf{[VII.24]}$$

where

$$\begin{aligned}
b_1 &= \frac{a_1a_2 - a_0a_3}{a_1}, b_2 = \frac{a_1a_4 - a_0a_5}{a_1}, b_3 = \frac{a_1a_6 - a_0a_7}{a_1} \\
c_1 &= \frac{b_1a_3 - a_1b_2}{b_1}, c_2 = \frac{b_1a_5 - a_1b_3}{b_1}, c_3 = \frac{b_1a_7 - a_1b_4}{b_1} \\
d_1 &= \frac{c_1b_2 - b_1c_2}{c_1}, d_2 = \frac{c_1b_3 - b_1c_3}{c_1}
\end{aligned} \qquad \textbf{[VII.25]}$$

The Routh-Hurwitz stability criterion states that the number of roots with positive real parts is equal to the number of sign changes of coefficients in the first column of the matrix. Let's apply this criterion to our problem. First we have to make sure that all coefficient a_i are positive. a_o and a_1 are always positive, a_4 is positive if:

$$g_{14}g_{32} < 1 - g_{12} \qquad \textbf{[VII.26]}$$

This is the line with the negative slope in the stability diagram (Fig. 1B). a_2 is positive if:

$$\beta_1\beta_2 + \beta_1\beta_3 + \beta_1\beta_4 + \beta_2\beta_3 + \beta_2\beta_4 + \beta_3\beta_4 > g_{12}\beta_1\beta_2 \qquad \textbf{[VII.27]}$$

If $\beta_1 \approx \beta_3$ and $\beta_2 \approx \beta_4$ this is satisfied when $g_{12} < 4$. Similarly, a_3 is positive if

$$\beta_1\beta_2\beta_3 + \beta_1\beta_2\beta_4 + \beta_2\beta_3\beta_4 + \beta_1\beta_3\beta_4 > g_{12}\left(\beta_1\beta_2\beta_3 + \beta_1\beta_2\beta_4\right) \qquad \textbf{[VII.28]}$$

If $\beta_1 \approx \beta_3$ and $\beta_2 \approx \beta_4$ this is satisfied when $g_{12} < 2$. Therefore the conditions for positive a_i are:

$$\begin{cases} g_{14}g_{32} < 1 - g_{12} \\ g_{12} < 2 \end{cases} \qquad \textbf{[VII.29]}$$

The next step is to calculate b_1, c_1, and d_1. Substitution in [VII.25] yields $d_1 = b_2 = a_4 > 0$ because of [VII.26]. $b_1 > 0$ is equivalent to:

$$\sum_{i\neq j}\beta_i\beta_j - \frac{\sum_{i\neq j\neq k}\beta_i\beta_j\beta_k}{\sum_i \beta_i} > g_{12}\beta_1\beta_2 + \frac{g_{12}\left(\beta_1\beta_2\beta_4 + \beta_1\beta_2\beta_3\right)}{\sum_i \beta_i} \qquad \textbf{[VII.30]}$$

Rewriting gives:

$$2\sum_{i\neq j\neq k}\beta_i\beta_j\beta_k + \sum_{i\neq j}\beta_i^2\beta_j > g_{12}\beta_1\beta_2\sum_i \beta_i + g_{12}\left(\beta_1\beta_2\beta_4 + \beta_1\beta_2\beta_3\right) \qquad \textbf{[VII.31]}$$

The first term is larger than the last term in [VII.31] cancel if $g_{12}<4$ which is already satisfied by [VII.29]. The remaining is:

$$\sum_{i\neq j}\beta_i^2\beta_j > g_{12}\beta_1\beta_2\sum_i \beta_i \qquad \textbf{[VII.32]}$$

The left sum has in total 12 terms whereas the right has four. So as long as $g_{12}<3$, $b_1>0$. The last condition to prove is: $c_1>0$.

$$c_1 = a_3 - \frac{a_1 a_4}{b_1} = a_3 - \frac{a_1^2 a_4}{a_1 a_2 - a_3} > 0 \qquad \textbf{[VII.33]}$$

Substitution of [VII.25] gives:

$$g_{14}g_{32} < 1 - g_{12} + \frac{1}{\beta_1\beta_2\beta_3\beta_4\left(\beta_1+\beta_2+\beta_3+\beta_4\right)^2}\left[a_1a_2a_3 - a_3^2\right] \qquad \textbf{[VII.34]}$$

The easiest way to solve this is graphically. The values for the degradation constants are: $\beta_1 = \beta_3 = \beta_5 = 20.8 + 0.696/t_d$ hr^{-1} and $\beta_2 = \beta_4 = \beta_6 = 0.696/t_d$ hr^{-1}. Figure 12 shows the stability region (also see MATLAB code 6).

MATLAB code 6: Routh-Hurwitz criterion:

```
clear;
close;
g1=0:0.1:4;

t_D=0.5;

b1=20.8+0.696/t_D;
b2=0.696/t_D;
b3=20.8+0.696/t_D;
b4=0.696/t_D;

a0=1;
a1=(b1+b2+b3+b4);
a2=(b1*b2+b1*b3+b1*b4+b2*b3+b2*b4+b3*b4)-g1*b1*b2;
a3=b1*b2*b3+b1*b2*b4+b2*b3*b4+b1*b3*b4-g1*b1*b2*b3-g1*b1*b2*b4;

y1=1-(a1*a2.*a3-a3.*a3)./(a1*a1*b1*b2*b3*b4);
y2=1-g1;

plot(g1,y1,'b',g1,y2,'r');
axis([0 4 -20 2]);
grid on;
xlabel('g12');
ylabel('g14g32');
```

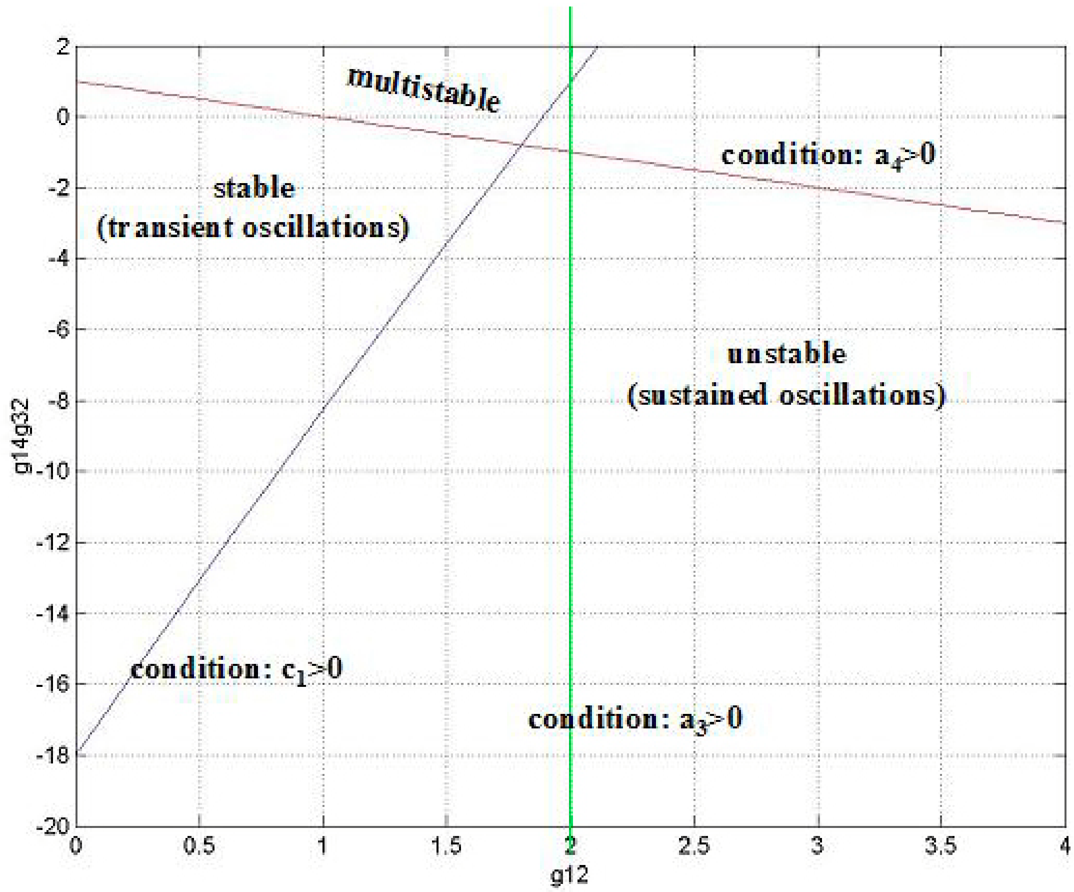

Figure 12. Stability analysis of synthetic oscillator of Atkinson et al.