Robustness of Synthetic Circadian Clocks to Multiple Environmental Changes

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Supporting information

1. Computational Procedures

Our computations were performed by running, in *Matlab*, the kinetics of Equations (1-3), shown in the manuscript, for i,j,k = 1,2,3, using rate constants corresponding to standard experimental conditions[1,2] (Table s1). For this purpose, we built a user-friendly graphical user interface (GUI, Scheme s2), which allows us to interactively adjust the initial concentrations and rate constants. This GUI also allows us to enter multiple inputs for any of the parameters, resulting in several simultaneous runs that are compared in one plot. When initially called, the GUI displays default parameter values, which may be adjusted. When the "Run" button is activated, the kinetics of Equations (1-3) are computed. First, the initial concentrations of the intermediates are calculated by running the reactions without ligation until equilibrium is reached. Then, all stages of the reactions are simultaneously computed by solving the differential equations using the Runge-Kutta method, over incremental time steps. The net production of the products t_i in all forms are monitored according to Equation (s1):

$$[t_i] = [T_i] + \sum_j [T_iT_j] + \sum_j [T_jT_i] + \sum_{j,k} [E_kNT_iT_j] + \sum_{j,k} [E_jNT_kT_i] + \sum_{j,k} [T_iT_jT_k] + \sum_{j,k} [T_kT_iT_j] + \sum_{j,k} [T_jT_kT_i] \quad \text{(s1)}$$

The following rate constant values were used for the 'default' environmental conditions: a_{ijk}, $\langle d \rangle_{jk}$ and $\langle f \rangle_{ijk}$, the diffusion constants, and f_{ijk}, the dissociation constants of the intermediate trimers $T_iT_jT_k$: 10^9, 10^6, 10^6 and 10^3, respectively, for allowed network connections, and zero for interrupted network connections; b_i, the reaction constants of the template-induced ligation: 10; g_i, the reaction constants of the background ligation: 1; d_{jk}, the dissociation constants of the intermediate dimers T_jT_k: 10; $\langle a \rangle_{ijk}$, the dissociation constants of the intermediates $E_iNT_jT_k$: 10.

The concentrations corresponding to the base parameters consisted of: E_2' = 25 μM, T_1 = 2.5 μM, N = 100 μM, $E_1 = E_3 = T_2 = T_3 = 0$. Two other sets of concentrations were used: one set used higher T_1 activator concentration of 10 μM; the other set used higher E_2' inhibitor concentration of 100 μM.

The 'basic' flux rate corresponded to E_3 and N intake rates of 10^{-4}, and a T_3 decay according to the following rate:

$$\frac{dT_3}{d(time)} = \frac{k_t T_3}{K_m + T_3} \qquad \text{(s2)}$$

where k_t = 0.12 and K_m = 0.01. The higher, 'basic' + 20% flux rate, corresponded to intake rates of $1.2 \cdot 10^{-4}$ and k_t = 0.144. All other intakes and decays were set to zero.

We simulated the different environmental conditions by systematically adjusting the rate constants of Equations (1-3) in the manuscript. The parameters for 'default' environmental conditions closely corresponded to our previously studied experimental and simulated systems,[1,2] typically operating at room temperature (25°C), pH 7, and natural salt concentrations. The following changes in rate constants were then chosen to reflect the reactions under varying environmental conditions (see also in Table s1). We note that in order to choose these parameters we sometime used qualitative rules-of-thumb practiced in the biochemistry field; some of the global changes made (e.g., flat multiplication of all rate constants) might be better fine-tuned by changing individual parameters differently.

- To study the system under high temperature (+10° C) we assumed a flat two-fold increase in all rate constants; low temperature (-10° C) assumed a two-fold decrease in all rate constants;
- Changes in salt concentrations are primarily assumed to affect the dissociation of template, substrate-template and product-template complexes. High salt conditions were therefore simulated by a decrease in dissociation rate constants $<a>_{ijk}$, f_{ijk} and d_{jk} by a factor of 1.2; low salt conditions assumed an increase in these dissociation rate constants by a factor of 1.5;
- Changes in pH (within a certain range) are expected to mainly affect the ligation reaction that transforms E_i and N to T_i. Therefore, high pH (8) assumed a ten-fold increase in the ligation rate constants b_i and g_i; low pH (6.3) assumed a five-fold decrease in these ligation rate constants; and very low pH (6) assumed a ten-fold decrease in these ligation rate constants.

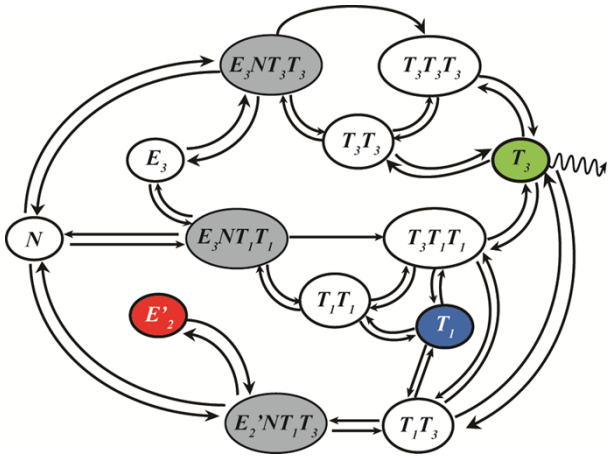
The period robustness has been calculated as the reciprocal of the product of the three ratios, comparing the periods of the oscillations from the two different fluxes, for the three sets of initial concentrations. Each of the ratios is defined as the maximum value divided by the minimum value, so the calculated robustness will always range from 0 to 1.

The sustainability of the oscillations over time has been calculated by observing the time-dependent behaviour of the oscillations, and computing the ratio of the amplitude of the final peak to the amplitude of the second peak.

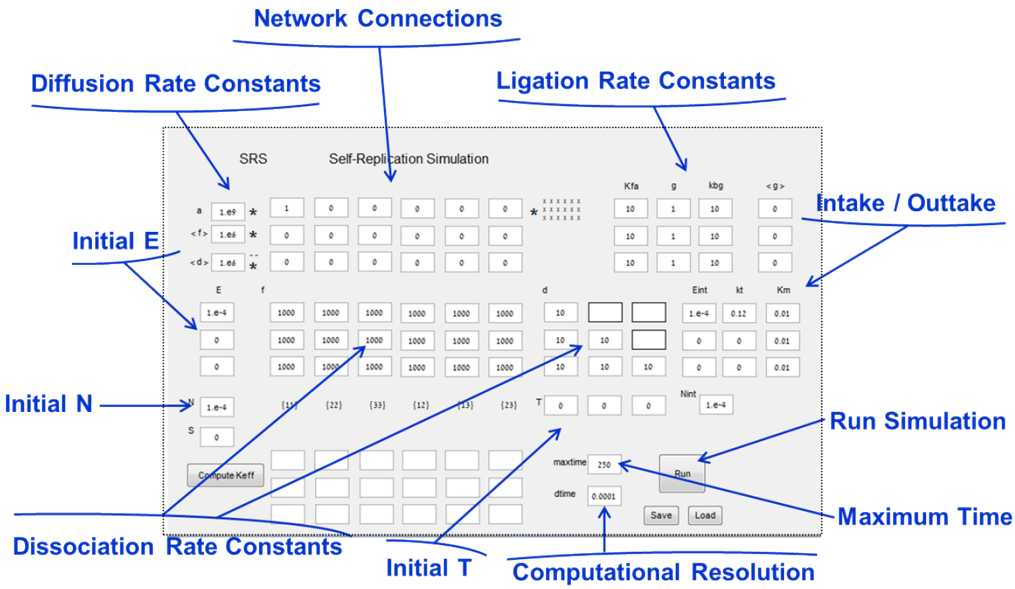
[1] N. Wagner and G. Ashkenasy, *Chem. Eur. J.* 2009, **15**, 1765.

[2] N. Wagner, S. Alasibi, E. Peacock-Lopez and G. Ashkenasy, *J. Phys. Chem. Lett.* 2015, **6**, 60.

2. Additional Schemes and Tables



Scheme s1. Kinetic diagram of the lead circadian network. The network function, robust oscillatory production of T_3, is governed by the following three reaction pathways: T_3 autocatalysis, T_3 production through cross catalysis by T_1, and a negative feedback loop due to formation of the E_2'NT_1T_3 inhibitory complex. The activator T_1, inhibitor E_2' and main intermediates formed along these pathways are highlighted.



Scheme s2. User-friendly graphical user interface (GUI), which allows interactive adjustment of the initial concentrations and rate constants. This GUI also allows us to enter multiple inputs for any of the parameters, resulting in several simultaneous runs that are compared in one plot. When initially called, the GUI displays default parameter values, which may be adjusted. When the "Run" button is activated, the kinetics of Equations (1-3) are then computed.

Table s1. Robustness by period (p) and sustainability (s) obtained from simulating the various network configurations under multiple environmental conditions, activator and inhibitor concentrations, and fluxes.

	Configuration -->	Native (I)		(II)		(III)		(IV)		(V)		
	Pathways [b] -->	a_{213}, a_{231}, a_{311}, a_{333}, f_{213}, f_{231}, f_{311}, f_{333}, $\langle f\rangle_{213}$, $\langle f\rangle_{231}$, $\langle f\rangle_{311}$, $\langle f\rangle_{333}$, $\langle d\rangle_{13}$, $\langle d\rangle_{31}$, $\langle d\rangle_{11}$, $\langle d\rangle_{33} \neq 0$		a_{311}, a_{333}, f_{311}, f_{333}, $\langle f\rangle_{311}$, $\langle f\rangle_{333}$, $\langle d\rangle_{11}$, $\langle d\rangle_{33} \neq 0$		a_{333}, f_{333}, $\langle f\rangle_{333}$, $\langle d\rangle_{33} \neq 0$		a_{211}, a_{311}, a_{333}, f_{211}, f_{311}, f_{333}, $\langle f\rangle_{211}$, $\langle f\rangle_{311}$, $\langle f\rangle_{333}$, $\langle d\rangle_{11}$, $\langle d\rangle_{33} \neq 0$		a_{233}, a_{311}, a_{333}, f_{233}, f_{311}, f_{333}, $\langle f\rangle_{233}$, $\langle f\rangle_{311}$, $\langle f\rangle_{333}$, $\langle d\rangle_{11}$, $\langle d\rangle_{33} \neq 0$		
Conditions [a]	**Flux** [d] -->	Low flux	High flux	Low flux	High flux	Low flux	High flux	Low flux	High flux	Low flux	High flux	**Conc.** [c]
Default conditions	$a=1\cdot10^9$, $\langle a\rangle = 10$, $\langle d\rangle=1\cdot10^6$, $\langle f\rangle=1\cdot10^6$, d=10, f=1000, g=1	P=1; S=1	P=1; S=1	P=0.92; S=1	P=0.92; S=1	P=2.41; S=1	P=2.28; S=1	P=0.95; S=1	P=0.95; S=1	P=0.76; S=0.86	P=0.74; S=0.89	Base
		P=0.61; S=0.71	P=0.59; S=0.8	P=0.56; S=0	P=0.53; S=0.11			P=0.56; S=0.14	P=0.55; S=0.56	P=0.29; S=0	P=0.56; S=0	High activator
		P=1.1; S=1	P=1.1; S=1					P=1.03; S=1	P=1.02; S=1	P=0.79; S=0	P=0.73; S=0	High inhibitor
High Temp	$a=2\cdot10^9$, $\langle a\rangle = 10$, $\langle d\rangle=2\cdot10^6$, $\langle f\rangle=2\cdot10^6$, d=20, f=2000, g=2	P=0.52; S=0.98	P=0.52; S=1	P=0.46; S=0.9	P=0.46; S=0.97	P=1.43; S=1	P=1.36; S=1	P=0.47; S=0.93	P=0.48; S=0.98	P=0.41; S=0	P=0.39; S=0.15	Base
		P=0.11; S=0	P=0.36; S=0	P=0.02; S=0	P=0.2; S=0			P=0.02; S=0	P=0.02; S=0	P=0.02; S=0	P=0.01; S=0	High activator
		P=0.59; S=1	P=0.58; S=1					P=0.52; S=0.97	P=0.53; S=0.99	P=0.01; S=0	P=0.01; S=0	High inhibitor
Low Temp	$a=0.5\cdot10^9$, $\langle a\rangle = 10$, $\langle d\rangle=0.5\cdot10^6$, $\langle f\rangle=0.5\cdot10^6$, d=5, f=500, g=0.5	P=1.84; S=0.99	P=1.74; S=0.99	P=1.73; S=0.99	P=1.64; S=0.98	P=2.84; S=1	P=2.28; S=1	P=1.77; S=0.99	P=1.67; S=0.98	P=1.35; S=0.88	P=1.28; S=0.85	Base
		P=1.12; S=0.87	P=1.12; S=0.86	P=1.03; S=0.73	P=1.03; S=0.76			P=1.07; S=0.8	P=1.07; S=0.8	P=1; S=0.05	P=0.98; S=0.01	High activator
		P=2.03; S=1	P=1.89; S=0.99					P=1.85; S=0.99	P=1.73; S=0.98	P=1.16; S=0	P=1.11; S=0	High inhibitor
High salt	$a=1\cdot10^9$, $\langle a\rangle = 8.3$, $\langle d\rangle=1\cdot10^6$, $\langle f\rangle=1\cdot10^6$, d=8.3, f=833, g=1	P=0.59; S=0.6	P=0.58; S=0.73	P=0.55; S=0.15	P=0.54; S=0.48	P=2.37; S=1	P=2.31; S=1	P=0.56; S=0.28	P=0.55; S=0.56	P=0.09; S=0	P=0.47; S=0	Base
		P=0.56; S=0	P=0.52; S=0	P=0.01; S=0	P=0.01; S=0			P=0.01; S=0	P=0.37; S=0	P=0.01; S=0	P=0.01; S=0	High activator
		P=0.62; S=0.7	P=0.61; S=0.79					P=0.58; S=0.48	P=0.57; S=0.66	P=0.02; S=0	P=0.01; S=0	High inhibitor
Low salt	$a=1\cdot10^9$, $\langle a\rangle = 15$, $\langle d\rangle=1\cdot10^6$, $\langle f\rangle=1\cdot10^6$, d=15, f=1500, g=1	P=1.31; S=1	P=1.32; S=1	P=1.27; S=1	P=1.28; S=1	P=2.45; S=1	P=2.37; S=1	P=1.28; S=1	P=1.3; S=1	P=1.29; S=1	P=1.3; S=1	Base
		P=0.8; S=1	P=0.78; S=1	P=0.75; S=1	P=0.74; S=1			P=0.76; S=1	P=0.75; S=1	P=0.77; S=1	P=0.75; S=1	High activator
		P=1.35; S=1	P=1.36; S=1					P=1.32; S=1	P=1.33; S=1	P=1.34; S=1	P=1.35; S=1	High inhibitor
High pH	$a=1\cdot10^9$, $\langle a\rangle = 10$, $\langle d\rangle=1\cdot10^6$, $\langle f\rangle=1\cdot10^6$, d=10, f=1000, g=10	P=0.46; S=1	P=0.43; S=1	P=0.4; S=0.94	P=0.38; S=0.98	P=0.83; S=1	P=0.77; S=1	P=0.42; S=0.97	P=0.4; S=0.99	P=0.38; S=0	P=0.35; S=0	Base
		P=0.04; S=0	P=0.32; S=0	P=0.01; S=0	P=0.01; S=0			P=0.01; S=0	P=0.01; S=0	P=0.02; S=0	P=0.01; S=0	High activator
		P=0.5; S=1	P=0.46; S=1					P=0.47; S=1	P=0.44; S=1	P=0.03; S=0	P=0.02; S=0	High inhibitor
Low pH	$a=1\cdot10^9$, $\langle a\rangle = 10$, $\langle d\rangle=1\cdot10^6$, $\langle f\rangle=1\cdot10^6$, d=10, f=1000, g=0.2	P=3.17; S=0.95	P=2.75; S=0.89	P=3.12; S=0.95	P=2.72; S=0.88	P=4.46; S=0.99	P=3.5; S=0.93	P=3.15; S=0.95	P=2.74; S=0.88	P=2.32; S=0.81	P=2.06; S=0.73	Base
		P=1.84; S=0.83	P=1.79; S=0.77	P=1.82; S=0.83	P=1.79; S=0.78			P=1.85; S=0.84	P=1.81; S=0.79	P=1.62; S=0.63	P=1.58; S=0.59	High activator
		P=3.38; S=0.96	P=2.19; S=0.91					P=3.23; S=0.95	P=0.79; S=0.89	P=1.57; S=0.13	P=1.5; S=0.11	High inhibitor
Very low pH	$a=1\cdot10^9$, $\langle a\rangle = 10$, $\langle d\rangle=1\cdot10^6$, $\langle f\rangle=1\cdot10^6$, d=10, f=1000, g=0.1	P=3.74; S=0.69	P=3.06; S=0.56	P=3.85; S=0.69	P=3.15; S=0.56	N/A	N/A	P=3.86; S=0.69	P=3.16; S=0.56	P=3.13; S=0.56	P=2.74; S=0.47	Base
		P=2.83; S=0.61	P=2.6; S=0.52	P=2.92; S=0.63	P=2.69; S=0.54			P=2.94; S=0.63	P=2.69; S=0.54	P=2.63; S=0.51	P=2.48; S=0.44	High activator
		P=3.87; S=0.71	P=3.12; S=0.57					P=3.89; S=0.69	P=3.16; S=0.56	P=2.49; S=0.28	P=2.35; S=0.22	High inhibitor

[a] in all condition sets – $N = 1\cdot10^{-4}$.

[b] For all configurations: $g_1 = g_2 = 0$, $g_3 \neq 0$, and $b_1 = b_2 = 0$, $b_3 = 10 \times g_3$.

[c] Base: $E_2' = 2.5\cdot10^{-5}$, $T_1 = 2.5\cdot10^{-6}$; High activator: $E_2' = 2.5\cdot10^{-5}$, $T_1 = 1\cdot10^{-5}$; High inhibitor: $E_2 = 1\cdot10^{-4}$, $T_1 = 2.5\cdot10^{-6}$.

[d] Low flux: E_3intake = Nintake = $1\cdot10^{-4}$, $k_t = 0.12$ for T_3 decay; High flux: E_3intake = Nintake = $1.2\cdot10^{-4}$, $k_t = 0.144$ for T_3 decay.