SUPPLEMENTARY MATERIAL

MATHEMATICAL MODEL OF THE DROSOPHILA CIRCADIAN CLOCK: LOOP REGULATION AND TRANSCRIPTIONAL INTEGRATION

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Parameters and Simulations

The oscillations of cry mRNA are modeled by linear elements that fit previously published microarray data (see Figure S1). We fix the decay rates $(\delta_i = -0.01)$; the saturation levels (s_i) are set at 100, except for:

1) $s_{PER/TIM-p} = \text{minimum}(\{x_{PER}, x_{TIM}, ([DBT]^* \lambda_{DBT,PER/TIM-p} * 500)\})$,

2) $s_{CLK/CYC} = \text{minimum}([CYC], x_{CLK})$, and

The latter and the remaining 41 parameters (see Matlab function and Table 1) are optimized to yield a numerical solution such that the molecules of the clock oscillate with a 24-hour period in LD and DD. The procedure is as follows; first, we find parameters that yield oscillations, then optimize by studying the effects of parameter perturbations on the following measures: 1) the period in LD and DD, and 2) timing of the peaks.

Modeling the Dynamics of the Opposing Transcriptional Signals

We use the symbol g to refer to the direct target genes *per*, *tim*, *vri*, *pdp1*, and *cwo*. Each direct target mRNA peaks at the same time (t_g) referenced to the peak time of CLK in both the wt and cwo-mutant models in LD (data not shown). Let Y_g and $Y_g^{\text{cwo-mutant}}$ denote the concentration levels at the peaks (maxima) of direct target genes in the wt and mutant models, respectively. Let $x_{C/C}(t)$ and $x_{C/C}^{\text{cwo-mutant}}(t)$ denote the concentration levels of CLK-CYC in the wt and mutant models, respectively. Let $x_{CWO}(t)$ be the concentration of CWO protein in the wt model. By equation (1),

$$\lambda_{C/C,g} x_{C/C}^{\text{cwo-mutant}}(t_g) - |\delta_g| Y_g^{\text{cwo-mutant}} = 0 \Leftrightarrow Y_g^{\text{cwo-mutant}} = \frac{\lambda_{C/C,g}}{|\delta_g|} x_{C/C}^{\text{cwo-mutant}}(t_g), \text{ and}$$

$$\lambda_{C/C,g} x_{C/C}(t_g) - |\lambda_{CWO,g}| x_{CWO}(t_g) - |\delta_g| Y_g = 0 \Leftrightarrow Y_g = \frac{\lambda_{C/C,g}}{|\delta_g|} x_{C/C}(t_g) - \frac{|\lambda_{CWO,g}|}{|\delta_g|} x_{CWO}(t_g),$$

Thus,

$$\Delta Y_g = Y_g - Y_g^{\text{cwo-mutant}} = \frac{\lambda_{C/C,g}}{|\delta_g|}\left[x_{C/C}(t_g) - x_{C/C}^{\text{cwo-mutant}}(t_g)\right] - \frac{|\lambda_{CWO,g}|}{|\delta_g|} x_{CWO}(t_g)$$

$$\Leftrightarrow \Delta Y_g = \frac{1}{|\delta_g|}\left[\Delta x_{C/C}(t_g)\lambda_{C/C,g} - x_{CWO}(t_g)|\lambda_{CWO,g}|\right],\ \Delta x_{C/C}(t_g) = x_{C/C}(t_g) - x_{C/C}^{\text{cwo-mutant}}(t_g),$$

Finally:

$$sign(\Delta Y_g) = sign\left[\Delta x_{C/C}(t_g)\lambda_{C/C,g} - x_{CWO}(t_g)\left|\lambda_{CWO,g}\right|\right]. \quad \textbf{(2)}$$

Matlab Functions

The Matlab function Dclockode includes the system of ODE; save it in the 'work' folder of Matlab. Please copy the content of Dcode and paste into the Matlab Command Window. The pdf copy of Dclockode details the equations and parameters.

$\rho_{clk} = +0.00895$	$\rho_{pdp1} = +0.01$	$\lambda_{CYC-CLK,tim} = +0.0121$	$\lambda_{PDP1,clk} = +0.0115$
$\rho_{CLK} = +0.02$	$\rho_{PDP1} = +0.03$	$\lambda_{tim,TIM} = +0.0115$	$\lambda_{CYC-CLK,vri} = +0.00384$
$\rho_{CLK/CYC} = +0.006$	$\rho_{vri} = +0.04$	$\lambda_{CRY,TIM} = -0.0000935$	$\lambda_{vri,VRI} = +0.01$
$\rho_{per} = +0.0455$	$\rho_{VRI} = +0.02$	$\lambda_{TIM,PER-TIM-p} = +0.035$	$\lambda_{vri,clk} = -0.0114$
$\rho_{PER} = +0.0156$	$\lambda_{clk,CLK} = +0.026$	$\lambda_{DBT,PER-TIM-p} = +0.001$	$\lambda_{CWO,cwo} = -0.028$
$\rho_{tim} = +0.0151$	$\lambda_{CLK,CYC-CLK} = +0.1747$	$\lambda_{PER-TIM-p,CYC-CLK} = -0.87$	$\lambda_{CWO,per} = -0.0235$
$\rho_{TIM} = +0.083$	$\lambda_{CYC,CYC-CLK} = +0.062$	$\lambda_{CLK-CYC,cwo} = +0.0025$	$\lambda_{CWO,tim} = -0.022$
$\rho_{PER/TIM-p} = +0.032$	$\lambda_{CYC-CLK,per} = +0.01134$	$\lambda_{cwo,CWO} = +0.008$	$\lambda_{CWO,pdp1} = -0.008$
$\rho_{cwo} = +0.076$	$\lambda_{per,PER} = +0.0077$	$\lambda_{CYC-CLK,pdp1} = +0.009$	$\lambda_{CWO,vri} = -0.002$
$\rho_{CWO} = +0.06$	$\lambda_{PER,PER-TIM-p} = +0.028$	$\lambda_{pdp1,PDP1} = +0.01262$	$\rho_{CRY} = +0.01$
			$\lambda_{cry,CRY} = +0.009$

Table 1. The parameters of the system shown in (1).

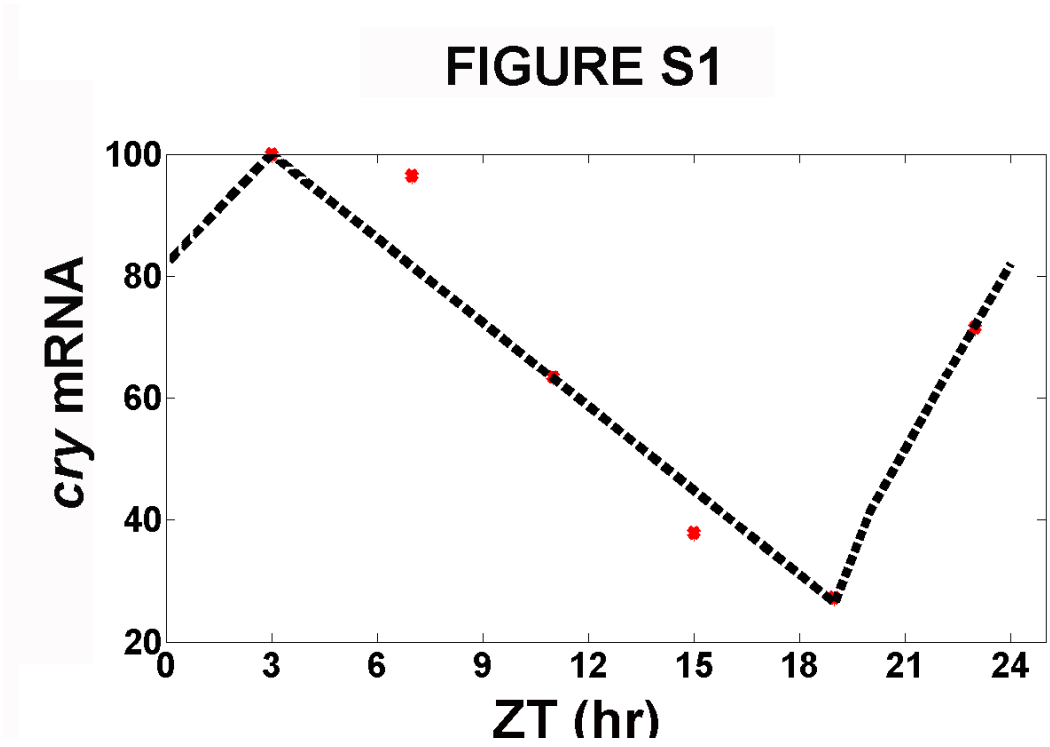

Figure S1. Microarray data of cry mRNA oscillations are fitted by linear elements.

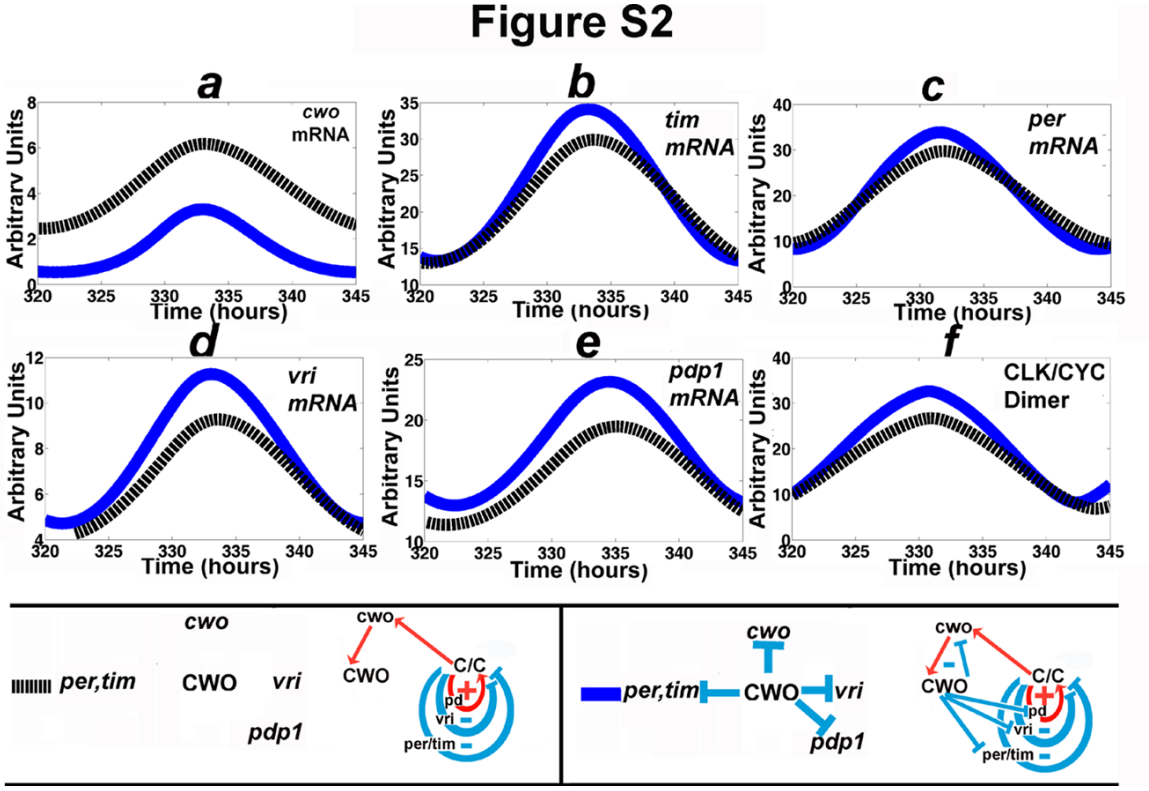

Figure S2. Modeling *cwo*-mutants in DD conditions. Each panel plots the mRNA concentration of a specific molecule (shown in the panel) in response to selective activation of the repressive actions of CWO in LD. Blue and dotted black lines represent the wt and mutant model, respectively.

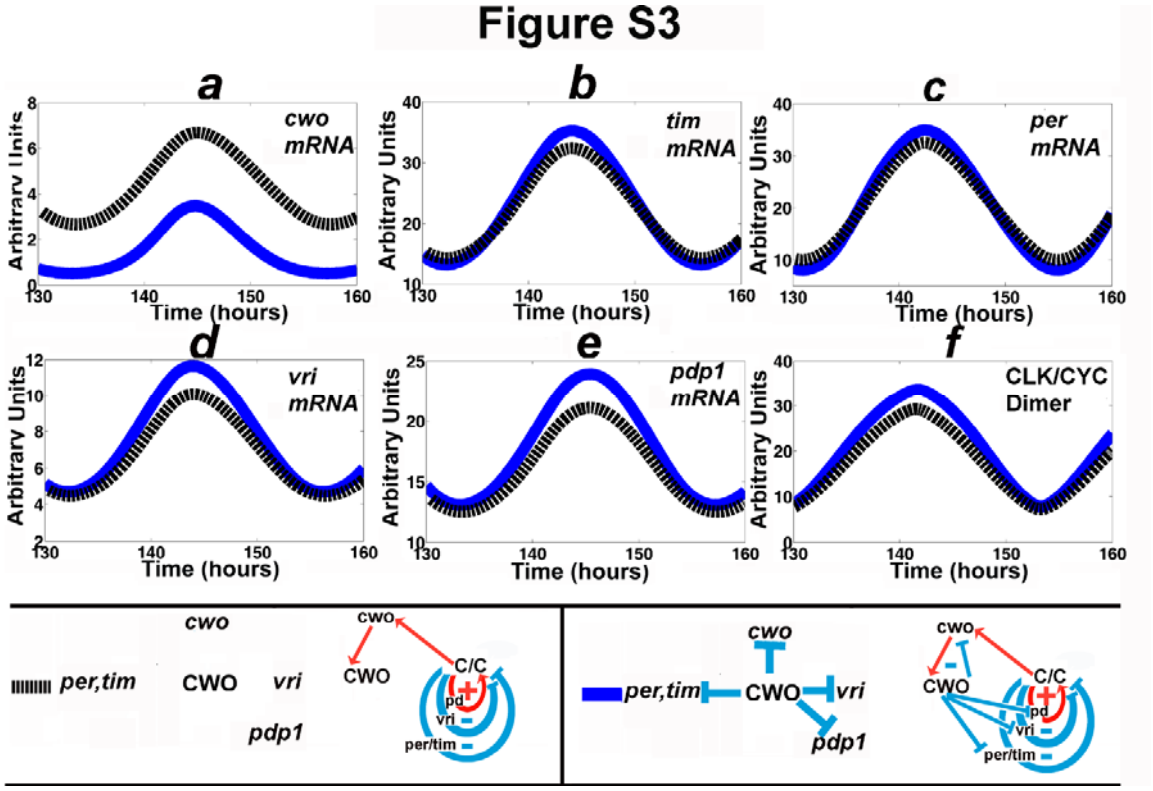

Figure S3. Modeling *cwo*-mutants in LD conditions. Each panel plots the mRNA concentration of a specific molecule (shown in the panel) in response to selective activation of the repressive actions of CWO in LD. Blue and dotted black lines represent the wt and mutant model, respectively.

FIGURE S4

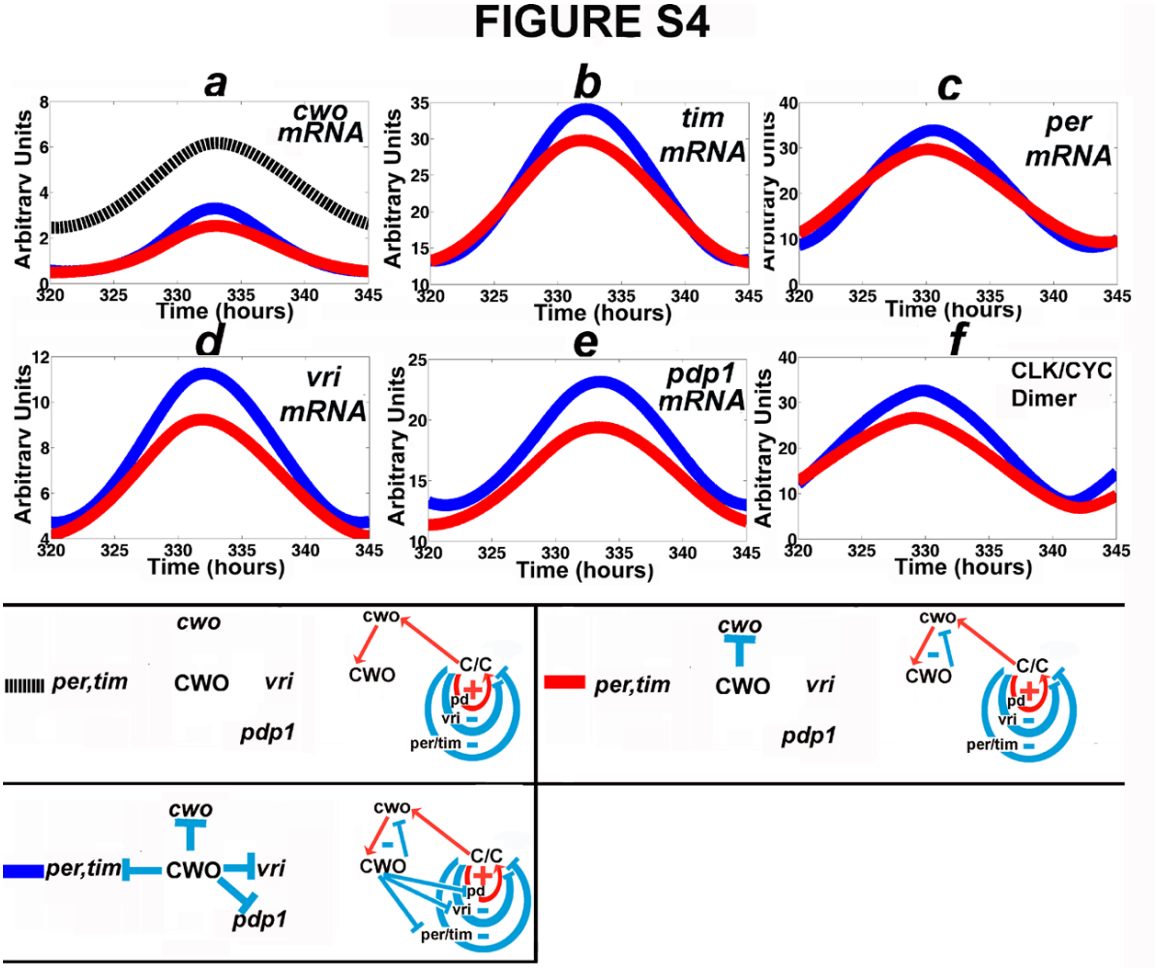

Figure S4. Actions of CWO on direct targets in DD conditions. This is a plot of the mRNA concentration of *cwo*, CLK-CYC and the other direct targets in response to selective activation of the repressive actions of CWO in DD. Blue and dotted black lines represent the wt and mutant model, respectively. The red line denotes the selective activation of the (*cwo*, CWO) auto-repressive loop only.

FIGURE S5

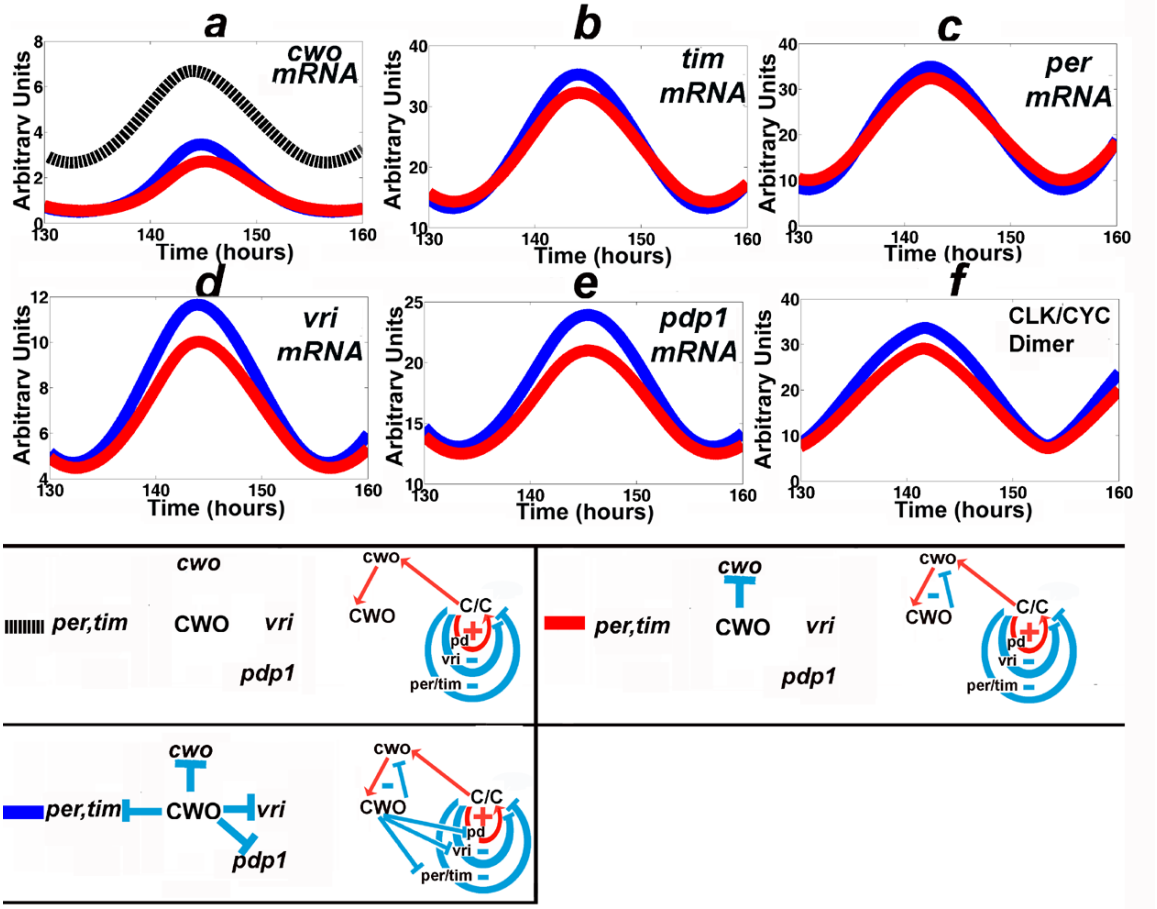

Figure S5. Actions of CWO on direct targets in LD conditions. This is a plot of the mRNA concentration of *cwo*, CLK-CYC and the other direct targets in response to selective activation of the repressive actions of CWO in LD. Blue and dotted black lines represent the wt and mutant model, respectively. The red line denotes the selective activation of the (*cwo*, CWO) auto-repressive loop only.

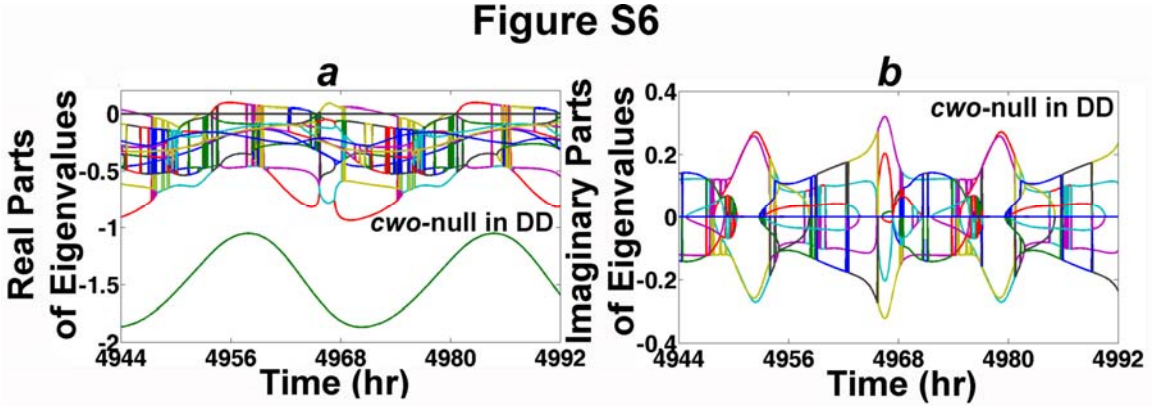

Figure S6. The Real and Imaginary Parts of the Eigenvalues in the *cwo*-mutant model. (*a*) and (*b*) plot the real and imaginary parts of the eigenvalues (different colors) of the flow matrix of the *cwo*-mutant model in DD conditions, respectively; observe that the real parts cross the *x*-axis. Time = 4920 hr corresponds to ZT = 0 hr.

```
%save Dclockode in "work" folder then
%copy below and paste into Matlab Command Window

hrs = 5000; %number of hours
x0 = [41.3307, 14.8523, 10.7140, 17.6771, 12.7573, 7.8339, 10.5919, 0.5072, ...
    2.1640, 12.0733, 15.7604, 4.7165, 6.4340, 13.3713, 73.9673]; % starting point
options = odeset('RelTol',1e-8,'AbsTol', [1e-16 1e-16 1e-16 1e-16 1e-16 1e-16 ...
    1e-16 1e-16 1e-16 1e-16 1e-16 1e-16 1e-16 1e-16 1e-16]);
[Ta,xa] = ode45(@Dclockode,[0 hrs],x0, options); %numerical integration


%Figure 1
%A graph of oscillations of all 15 molecules from t = 1 to time in LD
figure %figure 1
plot(Ta, xa)

%Figure 2
%A graph of oscillations of an individucal molecule, m, from t = 1 to time
%m = 1: CLK protein. m = 2: CLK/CYC dimer.
%m = 3: per mRNA. m = 4: PER protein.
%m = 5: tim mRNA. m = 6: TIM protein. m = 7: PER/TIM-P.
%m = 8: cwo mRNA. m = 9: CWO protein.
%m = 10: pdp1 mRNA. m = 11: PDP1 protein.
%m = 12: vri mRNA. m = 13: VRI protein.
%m = 14: clk mRNA. m = 15: CRY protein.

figure
m = 3; %per mRNA
plot(Ta, xa(:,m))
clear m

%Figure 3, mRNAs
%A graph of the relative abundance of direct target mRNAs
%per mRNA: blue; tim mRNA: red; cwo mRNA: yello;
%pdp1 mRNA: green; vri mRNA: dotted cyan.

s = length(Ta); d = 3000;
k = round((s/5000)* 30);
clear A a i j
for i = 1:k
    A(i) = mod(Ta(s - d + i),24);
end
j = find(A == min(A))

a = s - d + j,

b = a + round(length(Ta)/5000 * 27.5);
%a = 13450; b = 14100; %DD
c1 = min(xa(a:b,1)); d1 = max(xa(a:b,1));
c2 = min(xa(a:b,2)); d2 = max(xa(a:b,2));
c3 = min(xa(a:b,3)); d3 = max(xa(a:b,3));
c4 = min(xa(a:b,4)); d4 = max(xa(a:b,4));
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```
c5 = min(xa(a:b,5)); d5 = max(xa(a:b,5));
c6 = min(xa(a:b,6)); d6 = max(xa(a:b,6));
c7 = min(xa(a:b,7)); d7 = max(xa(a:b,7));
c8 = min(xa(a:b,8)); d8 = max(xa(a:b,8));
c9 = min(xa(a:b,9)); d9 = max(xa(a:b,9));

c10 = min(xa(a:b,10)); d10 = max(xa(a:b,10));
c11 = min(xa(a:b,11)); d11 = max(xa(a:b,11));
c12 = min(xa(a:b,12)); d12 = max(xa(a:b,12));
c13 = min(xa(a:b,13)); d13 = max(xa(a:b,13));
c14 = min(xa(a:b,14)); d14 = max(xa(a:b,14));

TT = Ta(a) - mod(Ta(a), 24);
c = round(mod(Ta(a), 24));

%A graph of the relative abundance of mRNAS
%per mRNA: blue; tim mRNA: red; cwo mRNA: yello;
%pdp1 mRNA: green; vri mRNA: dotted cyan.

figure %Figure 2a
plot(Ta(a:b) - Ta(a),(xa(a:b,3) - c3) ./ (d3 - c3),'-b', ...
    Ta(a:b) - Ta(a),(xa(a:b,5) - c5)./ (d5 - c5),'-r', ...
    Ta(a:b) - Ta(a), (xa(a:b,8) - c8)./(d8-c8),'-y',...
    Ta(a:b)- Ta(a),(xa(a:b,14) - c14)./(d14-c14),'-k', ...
    Ta(a:b) - Ta(a),(xa(a:b,10) - c10) ./ (d10-c10),'-g', ...
    Ta(a:b) - Ta(a),(xa(a:b,12) - c12)./ (d12 - c12),'--c', ...
    'LineWidth', 8)


%A graph of the relative abundance of Proteins
%PER: blue; TIM: red; CWO: yello;
%PDP!: green; VRI: dotted cyan.

figure  %Fig 2b, PROTEIN in LD
plot(Ta(a:b) - Ta(a),(xa(a:b,4) - c4) ./ (d4 - c4),'-b', ...
    Ta(a:b) - Ta(a),(xa(a:b,6) - c6)./ (d6 - c6),'-r', ...
    Ta(a:b) - Ta(a), (xa(a:b,9) - c9)./(d9-c9),'-y',...
    Ta(a:b)- Ta(a),(xa(a:b,1) - c1)./(d1-c1),'-k', ...
    Ta(a:b) - Ta(a),(xa(a:b,11) - c11) ./ (d11-c11),'-g', ...
    Ta(a:b) - Ta(a),(xa(a:b,13) - c13)./ (d13 - c13),'--c', ...
    'LineWidth', 8)
```

```
function dx = Dclockode(t,x)



%ode function for the molecular network of the Drosophila circadian clock.
%Save this file in the 'work' folder.
%x(1) = CLK protein; x(2) = CLK/CYC dimer;
%x(3) = per mRNA; x(4) = PER protein;
%x(5) = tim mRNA; x(6) = TIM protein; x(7) = PER/TIM-P;
%x(8) = cwo mRNA; x(9) = CWO protein;
%x(10) = pdp1 mRNA; x(11) = PDP1 protein;
%x(12) = vri mRNA; x(13) = VRI protein;
%x(14) = clk mRNA; x(15) = CRY protein.

%Parameters ri indicating maximal rates of formation of x(i)
r1 = 0.02;
r2 = 0.006;
r3 = 0.0455;
r4 = 0.0156;
r5 = 0.0151;
r6 = 0.083;
r7 = 0.032;
r8 = 0.076;
r9 = 0.06;
r10 = 0.01;
r11 = 0.03;
r12 = 0.04;
r13 = 0.02;
r14 = 0.00895;
r15 = 0.01;

%SATURATION PARAMETERS
S1 = 100;
S2 = 100;
S3 = 100;
S4 = 100;
S5 = 100;
S6 = 100;
S7 = 100;
S8 = 100;
S9 = 100;
S10 = 100;
S11 = 100;
S12 = 100;
S13 = 100;
S14 = 100;
S15 = 100;

%DEGRADATION PARAMETERS
d1 = -0.01;
d2 = -0.01;
d3 = -0.01;
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d4 = -0.01;
d5 = -0.01;
d6 = -0.01;
d7 = -0.01;
d8 = -0.01;
d9 = -0.01;
d10 = -0.01;
d11 = -0.01;
d12 = -0.01;
d13 = -0.01;
d14 = -0.01;
d15 = -0.01;

%REGULATORY WEIGHTS
T141 = 0.026; %clk mRNA regulates CLK PROTEIN
T12 = 0.1747; %CLK PROTEIN regulates CLK-CYC dimer
Y = 100;  % Concentration of CYC (arbitrary units)
Ty2 = 0.062; %CYC protein regulates CLK-CYC dimer
T23 = 0.01134; %CLK/CYC regulates per mRNA
T34 = 0.0077; %per mRNA regulates PER protein
T47 = 0.028; %per protein regulates PER-TIM-P
T25 = 0.0124; %CLK-CYC regulates tim mRNA
T56 = 0.0115; %tim mRNA regulates TIM protein
T67 = 0.035; %TIM protein regulates PER/TIM-P
xp = 100; %DBT concentration (arbitrary units)
Tp7 = 0.001; % DBT-mediated Phosphorylation of PER-TIM leading to PER-TIM-P
T72 = -0.87; %PER-TIM-P negatively regulates CLK-CYC activity

T210 = 0.009; %CLK/CYC regulates pdp1 mRNA
T1011 = 0.01262; %pdp1 mRNA regulates PDP1 prtein
T1114 = 0.0115; %PDP1 protein positively regulates clk mRNA
T212 = 0.00384; %CLK/CYC regulates vri mRNA
T1213 = 0.01; %vri mRNA regulates VRI protein
T1314 = -0.0114; %VRI protein negatively regulates clk mRNA
Tcry = 0.009; %cry mRNA regulates CRY protein
Tcry6 = -0.0000935; %CRY protein degrades TIM protein

T28 = 0.0025; %CLK/CYC regulates cwo mRNA
T89 = 0.008; %cwo mRNA regulates CWO protein
T93 = -0.0235; %CWO protein negative regulation of per mRNA
T95 = -0.022; %CWO protein negative regulation of tim mRNA
T98 = -0.028; %CWO protein negative regulation of cwo mRNA
T910 = -0.008; %CWO protein negative regulation of pdp1 mRNA
T912 = -0.002; %CWO protein negative regulation of vri mRNA

%%simulation of cry mRNA oscillations based on microarray data
tt = mod(t, 24);
a = tt <= 3;
F1 = (((100 - 82)/3) .* tt + 82) .* a;
b = tt <= 19 & tt > 3;
F2 = ((-(100 - 31)/15) .* (tt-3) + 100) .* b ;
c = tt > 19;
```

```
F3 = (((72 - 31)/4) .* (tt - 23) + 72) .* c;
cry = F1 + F2 + F3;  %cry mRNA

%SYSTEM OF ODE
dx = zeros(size(x));

dx(1) = r1 * ((T141 * x(14) + d1 * x(1)) / ...
    sqrt(1 + ((T141 * x(14) + d1 * x(1)) ^2))) ...
    * x(1) * (S1 - x(1));

dx(2) = r2 * ((T12 * x(1) + Ty2 * Y + T72 * x(7) + d2 * x(2)) / ...
    sqrt(1 + ((T12 * x(1) + Ty2 * Y + T72 * x(7) + d2 * x(2)) ^2))) ...
    * x(2) * (1/2*(Y + x(1) - abs(Y - x(1))) - x(2));

dx(3) = r3 * ((T23 * x(2) + T93 * x(9) + d3 * x(3)) / ...
    sqrt(1 + ((T23 * x(2) + T93 * x(9) + d3 * x(3)) ^2))) ...
    * x(3) * (S3 - x(3));

dx(4) = r4 * ((T34 * x(3) + d4 * x(4)) / ...
    sqrt(1 + ((T34 * x(3) + d4 * x(4)) ^2))) ...
    * x(4) * (S4 - x(4));

dx(5) = r5 * ((T25 * x(2) + T95 * x(9) + d5 * x(5)) / ...
    sqrt(1 + ((T25 * x(2) + T95 * x(9) + d5 * x(5)) ^2))) ...
    * x(5) * (S5 - x(5));

dx(6) = r6 * ((T56 * x(5) + (Tcry6 .* x(15) + d6) * x(6)) / ...
    sqrt(1 + ((T56 * x(5) + (Tcry6 .* x(15) + d6) * x(6)) ^2))) ...
    * x(6) * (S6 - x(6));

dx(7) = r7 * ((Tp7* xp + T47 * x(4) + T67 * x(6) + d7 * x(7)) / ...
    sqrt(1 + ((Tp7 * xp + T47 * x(4) + T67 * x(6) + d7 * x(7)) ^2))) ...
    * x(7) * (1/2*(1/2*(x(4) + x(6) - abs(x(4) - x(6))) + (Tp7 * xp * 50/(100 * 0.001))↙
...
    - abs(1/2*(x(4) + x(6) - abs(x(4) - x(6))) - (Tp7 * xp * 50/(100 * 0.001)))) - x(7));

dx(8) = r8 * ((T28 * x(2) + T98 * x(9) + d8 * x(8)) / ...
    sqrt(1 + ((T28 * x(2) + T98 * x(9) + d8 * x(8)) ^2))) ...
    * x(8) * (S8 - x(8));

dx(9) = r9 * ((T89 * x(8) + d9 * x(9)) / ...
    sqrt(1 + ((T89 * x(8) + d9 * x(9)) ^2))) ...
    * x(9) * (S9 - x(9));

dx(10) = r10 * ((T210 * x(2) + T910 * x(9) + d10 * x(10)) / ...
    sqrt(1 + ((T210 * x(2) + T910 * x(9) + d10 * x(10)) ^2))) ...
    * x(10) * (S10 - x(10));

dx(11) = r11 * ((T1011 * x(10) + d11 * x(11)) / ...
    sqrt(1 + ((T1011 * x(10) + d11 * x(11)) ^2))) ...
    * x(11) * (S11 - x(11));
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dx(12) = r12 * ((T212 * x(2) + T912 * x(9) + d12 * x(12)) / ...
    sqrt(1 + ((T212 * x(2) + T912 * x(9) + d12 * x(12)) ^2))) ...
    * x(12) * (S12 - x(12));

dx(13) = r13 * ((T1213 * x(12) + d13 * x(13)) / ...
    sqrt(1 + ((T1213 * x(12) + d13 * x(13)) ^2))) ...
    * x(13) * (S13 - x(13));

dx(14) = r14 * ((T1114 * x(11) + T1314 * x(13) + d14 * x(14)) / ...
    sqrt(1 + ((T1114 * x(11) + T1314 * x(13) + d14 * x(14)) ^2))) ...
    * x(14) * (S14 - x(14));

dx(15) = r15 * (Tcry * cry + d15 * x(15)) / ...
    sqrt( 1 + ((Tcry * cry + d15 * x(15)) ^2)) ...
    * x(15) * (S15 - x(15));
```