Supplementary files

Branch point control at malonyl-CoA node: A computational framework to uncover the design principles of an ideal genetic-metabolic switch

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Parameters used for Fig. 1 to Fig. 10

Parameters for Fig. 2, Fig. 3 and Fig. 4: alpha1=0.8; alpha2=0.05; alpha3=0.05; alpha4=0.8; beta1=0.5; beta2=2.0; m=4; n=4; p=4; q=4; r=4; u=4; K1=2; K2=5; K3=2; K4=2; K5=0.5; K6=0.5; k1=0.5; k2=0.6; k3=2; k4=2; X0=0; S0=45; Y_XS=0.6; Y_PS1=0.4; Y_PS2=1.8; mu_max=2.2; K_S=0.75; K_m=0.5;

Parameters for Fig. 5 and Fig. 6: alpha1=0.8; alpha2=0.05; alpha3=0.05; alpha4=0.8; beta1=0.5; beta2=2.0; m=4; n=4; p=4; q=4; r=4; u=4; D=0.15; K2=5; K3=2; K4=2; K5=0.5; K6=0.5; k1=0.5; k2=0.6; k3=2; k4=2; X0=0; S0=45; Y_XS=0.6; Y_PS1=0.4; Y_PS2=1.8; mu_max=2.2; K_S=0.75; K_m=0.5;

Parameters for Fig. 7: alpha1=0.8; alpha2=0.05; alpha3=0.05; alpha4=0.8; beta1=0.5; m=4; n=4; p=4; q=4; r=4; u=4; D=0.15; K1=2; K2=5; K3=2; K4=2; K5=0.5; K6=0.5; k1=0.5; k2=0.6; k3=2; k4=2; X0=0; S0=45; Y_XS=0.6; Y_PS1=0.4; Y_PS2=1.8; mu_max=2.2; K_S=0.75; K_m=0.5;

Parameters for Fig. 8: alpha1=0.8; alpha2=0.05; alpha3=0.05; alpha4=0.8; beta1=0.5; beta2=2.0; m=4; n=4; p=4; q=4; r=4; u=4; D=0.15; K1=2; K2=5; K3=2; K5=0.5; K6=0.5; k1=0.5; k2=0.6; k3=2; k4=2; X0=0; S0=45; Y_XS=0.6; Y_PS1=0.4; Y_PS2=1.8; mu_max=2.2; K_S=0.75; K_m=0.5;

Parameters for Fig. 9: alpha1=0.8; alpha2=0.05; alpha3=0.05; alpha4=0.8; beta1=0.5; beta2=2.0; m=4; n=4; p=4; q=4; r=4; u=4; D=0.15; K1=2; K2=5; K4=2; K5=0.5; K6=0.5; k1=0.5; k2=0.6; k3=2; k4=2; X0=0; S0=45; Y_XS=0.6; Y_PS1=0.4; Y_PS2=1.8; mu_max=2.2; K_S=0.75; K_m=0.5;

Parameters for Fig. 10: alpha1=0.8; alpha2=0.05; alpha3=0.05; alpha4=0.8; beta1=0.5; beta2=2.0; m=4; p=4; q=4; r=4; u=4; D=0.15; K1=2; K2=5; K3=2; K4=2; K5=0.5; K6=0.5; k1=0.5; k2=0.6; k3=2; k4=2; X0=0; S0=45; Y_XS=0.6; Y_PS1=0.4; Y_PS2=1.8; mu_max=2.2; K_S=0.75; K_m=0.5;

Supplementary Figures

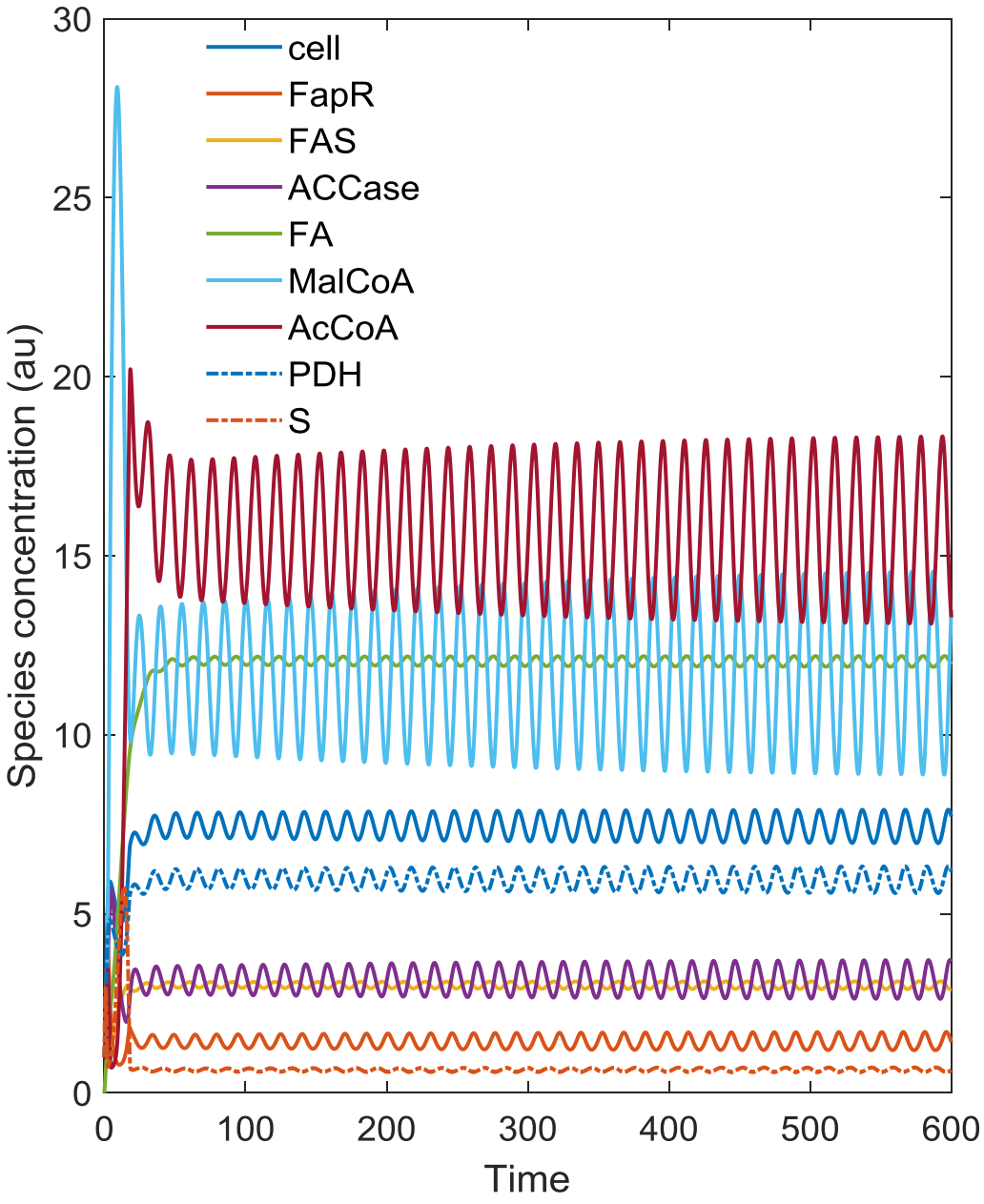


Fig. S1. Oscillatory pattern of species concentration for the autonomous Malonyl-CoA controller at D = 0.15. Other parameters used here are the same as the parameters used in Fig. 2.

Supplementary notes 1. Numerically evaluating the Jacobian Matrix and eigenvalues for the steady states (equilibrium points) listed in Fig. 2 and Fig. S1.

Here is a numerical evaluation of the Jacobian matrix and the corresponding eigenvalues for the equilibrium states in Fig. 2 (*D*=0.15) and Fig. S1. All MATLAB code and output results are marked in blue color.

```
>> D = 0.15;
tspan = [0:0.1:600];
y1_0=1; y2_0=1; y3_0=1; y4_0=1.0; y5_0=0; y6_0=1; y7_0=1; y8_0=1;
y9_0=1;
[T,Y]= ode45(@(t,y) MalCoA_Switch(t,y,D),tspan, [y1_0 y2_0 y3_0 y4_0 y5_0...
    y6_0 y7_0 y8_0 y9_0]);
plot(T,Y(:,1),'linewidth',1.5);
hold on
plot(T,Y(:,2),'-','linewidth',1.5);
plot(T,Y(:,3),'-','linewidth',1.5);
plot(T,Y(:,4),'-','linewidth',1.5);
plot(T,Y(:,5),'-','linewidth',1.5);
plot(T,Y(:,6),'-','linewidth',1.5);
plot(T,Y(:,7),'-','linewidth',1.5);
plot(T,Y(:,8),'-.','linewidth',1.5);
plot(T,Y(:,9),'-.','linewidth',1.5);
legend('cell','FapR','FAS','ACCase','FA','MalCoA','AcCoA','PDH','S');
legend boxoff;
xlabel('Time','FontSize',16);
ylabel('Species concentration (au)','FontSize',16);
>> J1 = numeric_jacobian(@test_MalCoA_Switch,Y(5838,:))
```

% Here is the Jacobian matrices numerically computed by Matlab. The equilibrium points are approximately taken at t=583.8, t=599.0 and t = 599.1. Note that we cannot accurately retrieve the steady states due to the limitations of numerical footstep (In ODE45, we iteratively retrieve a solution every 0.1 time scale). If we could take infinitesimally small footsteps, we may approach the exact equilibrium points.

```
J1 =

   -0.0118         0         0         0         0   -0.0760         0         0    0.9786
    0.1106   -0.6367         0         0         0   -0.0672         0         0    0.7829
    0.0069   -0.2491   -0.1500         0         0   -0.0038         0         0    0.0489
    0.0069    0.9962         0   -0.1500         0   -0.0038         0         0    0.0489
         0         0    0.6000         0   -0.1500    0.0000         0         0         0
         0         0   -1.5000    2.0000         0   -0.1500    0.0000         0         0
         0         0         0   -2.0000         0         0   -0.1500    1.3818   17.5659
    0.1106         0         0         0         0   -0.0608         0   -0.1500    0.7829
   -0.2304         0         0         0         0    0.1266         0   -0.7677  -11.5399

>> J2 = numeric_jacobian(@test_MalCoA_Switch,Y(5990,:))

J2 =

   -0.0114         0         0         0         0   -0.0764         0         0    0.9826
    0.1108   -0.6365         0         0         0   -0.0677         0         0    0.7861
    0.0069   -0.2497   -0.1500         0         0   -0.0038         0         0    0.0491
    0.0069    0.9987         0   -0.1500         0   -0.0038         0         0    0.0491
         0         0    0.6000         0   -0.1500    0.0000         0         0         0
         0         0   -1.5000    2.0000         0   -0.1500    0.0000         0         0
         0         0         0   -2.0000         0         0   -0.1500    1.3805   17.6082
    0.1108         0         0         0         0   -0.0611         0   -0.1500    0.7861
   -0.2309         0         0         0         0    0.1273         0   -0.7669  -11.5700

>> J3 = numeric_jacobian(@test_MalCoA_Switch,Y(5991,:))

J3 =

   -0.0124         0         0         0         0   -0.0752         0         0    0.9701
    0.1101   -0.6370         0         0         0   -0.0664         0         0    0.7761
    0.0069   -0.2480   -0.1500         0         0   -0.0038         0         0    0.0485
    0.0069    0.9920         0   -0.1500         0   -0.0038         0         0    0.0485
         0         0    0.6000         0   -0.1500    0.0000         0         0         0
         0         0   -1.5000    2.0000         0   -0.1500    0.0000         0         0
         0         0         0   -2.0000         0         0   -0.1500    1.3863   17.4373
    0.1101         0         0         0         0   -0.0601         0   -0.1500    0.7761
   -0.2294         0         0         0         0    0.1253         0   -0.7701  -11.4542
```

% We will numerically evaluate the eigenvalues of the Jacobian Matrices at the three approximately steady states at t=583.8, t=599.0 and t = 599.1, which are equivalently to the solution Y(5838,:), Y(5990,:) and Y(5991,:).

```
>> eig1=eig(J1)

eig1 =

  -0.1500 + 0.0000i
 -11.4679 + 0.0000i
  -0.8774 + 0.0000i
  -0.0002 + 0.4121i
  -0.0002 - 0.4121i
  -0.1428 + 0.0000i
  -0.1500 + 0.0000i
  -0.1500 + 0.0000i
  -0.1500 + 0.0000i

>> eig2=eig(J2)
```

```
eig2 =

  -0.1500 + 0.0000i
 -11.4979 + 0.0000i
  -0.8787 + 0.0000i
   0.0006 + 0.4137i
   0.0006 - 0.4137i
  -0.1427 + 0.0000i
  -0.1500 + 0.0000i
  -0.1500 + 0.0000i
  -0.1500 + 0.0000i

>> eig3=eig(J3)

eig3 =

  -0.1500 + 0.0000i
 -11.3822 + 0.0000i
  -0.8751 + 0.0000i
  -0.0017 + 0.4094i
  -0.0017 - 0.4094i
  -0.1430 + 0.0000i
  -0.1500 + 0.0000i
  -0.1500 + 0.0000i
  -0.1500 + 0.0000i
```

By evaluating the three steady states, we observed 7 of the 9 eigenvalues are negative real numbers in eig1, eig2 and eig3, except for the 4th and 5th components with imaginary parts which are highlighted in yellow. The highlighted eigenvalues for the three steady states have real parts approximating to zero with conjugate imaginary parts. For the 2nd steady state at t =599.0 and the 3rd steady state at t=599.1, we observed that the real part of the eigenvalues for J2 and J3 changed the sign. For example, the 4th and 5th components of the real part of eigenvalues changes its sign from +0.0006 in eig2 to -0.0017 in eig3, indicating that there exists a zero real part eigenvalue if we could take infinitesimally small footsteps between t=599.0 and t=599.1. Zero real part with imaginary eigenvalues indicate a stable oscillation. *Therefore, the steady state solutions are attracted to a limit cycle in the phase plane*.

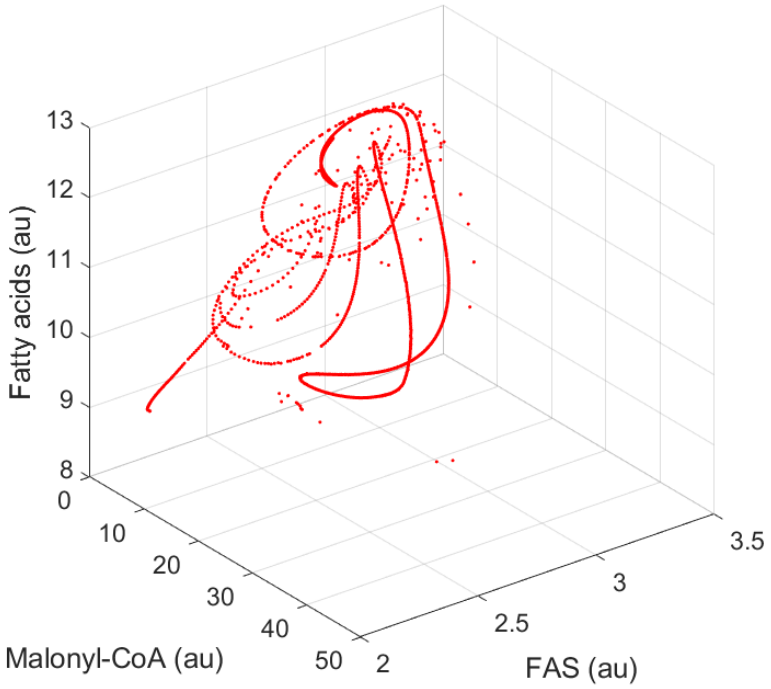
Supplementary notes 2. Plotting the steady state solutions of fatty acids, FAS and malonyl-CoA when the malonyl-CoA inhibition constant was changed.

By plotting the steady state solutions on a 3-D phase plane, we did find looping patterns, as we change the bifurcation parameter K1 (malonyl-CoA inhibition constant). Here is the code we run,

```
for K1=0.1:0.0005:1.5;
tspan = [0:0.1:120];
y1_0=9.4338; y2_0=4.2121; y3_0=2.3945; y4_0=6.1138; y5_0=11.1925;
y6_0=11.3272; y7_0=12.2715; y8_0=7.5470;
y9_0=0.6210;

[T,Y]= ode45(@(t,y) MalCoA_Switch_K1(t,y,K1),tspan, [y1_0 y2_0 y3_0 y4_0 y5_0...
    y6_0 y7_0 y8_0 y9_0]);
plot3(Y(1200,3),Y(1200,6),Y(1200,5), 'r.');
hold on;
end
>> xlabel('FAS (au)');
>> ylabel('Malonyl-CoA (au)');
>> zlabel('Fatty acids (au)');
```

% The initial condition starts with one of the equilibrium points when K1 = 0.5. Here is the 3-D phase plane when the malonyl-CoA inhibition constant (K1) was varied from 0.1 to 1.5. This figure was also attached as a main figure in the main text (Fig. 7). The looping pattern of steady state solutions (fatty acids, fatty acid synthase and malonyl-CoA) may indicate multiplicity and hysteresis of the system when K1 was changed.



Supplementary notes 3. Adding the trajectory with K1 = 0.3 to the 3-D phase plane.

```
for K1=0.1:0.0005:1.5;
tspan = [0:0.1:120];
y1_0=9.4338; y2_0=4.2121; y3_0=2.3945; y4_0=6.1138; y5_0=11.1925;
y6_0=11.3272; y7_0=12.2715; y8_0=7.5470; y9_0=0.6210;
[T,Y]= ode45(@(t,y) MalCoA_Switch_K1(t,y,K1),tspan, [y1_0 y2_0 y3_0 y4_0 y5_0...
    y6_0 y7_0 y8_0 y9_0]);
plot3(Y(1200,3),Y(1200,6),Y(1200,5), 'r.');
hold on
end
xlabel('FAS (au)');
ylabel('Malonyl-CoA (au)');
zlabel('Fatty acids (au)');
hold on
% draw a specific trajectory with K1=0.3 and a different starting point
K1=0.3;
tspan = [0:0.1:120];
y1_0=1; y2_0=1; y3_0=1; y4_0=1; y5_0=1; y6_0=1; y7_0=1; y8_0=1;
y9_0=1;
[T,Y]= ode45(@(t,y) MalCoA_Switch_K1(t,y,K1),tspan, [y1_0 y2_0 y3_0 y4_0 y5_0...
    y6_0 y7_0 y8_0 y9_0]);
plot3(Y(:,3),Y(:,6),Y(:,5), 'b.');

% A specific trajectory for K1 = 0.3 was added to the above solution space, marked in blue color in the figure attached below. This figure is attached as supplementary figures Fig. S2.
```