

S2 Text: Analytical derivation of steady states in one dimensional ODE for a reduced mFAO model

In order to analytically investigate bistability, we considered a reduced mFAO pathway around C4-KetoAcylCoA. The ODE related to the rate of change in the concentration C4-Ketoacyl-CoA over time is given by

$$VMAT \frac{dC4KetoacylCoAMAT}{dt} = vMSCHADC4 - vMCKATC4$$

where the rate equations are given by

$$vMSCHADC4 = \frac{sf_{vmschad}V_{max,mschad}}{\left(1 + \frac{[C4HydroxyacylCoA]}{Km_{MSCHAD,C4hydroxylCoA}} + \underbrace{\frac{[C4KetoacylCoA]}{Km_{MSCHAD,C4hydroxylCoA}}}_{inhibition\ by\ reaction\ product} + \sum_{n=6}^{16} \left\{ \frac{[CnHydroxyacylCoA]}{Km_{MSCHAD,CnhydroxylCoA}} + \frac{[CnKetoacylCoA]}{Km_{MSCHAD,CnketoacylCoA}} \right\} \right) \left(1 + \frac{[Nt - NADH]}{Km_N} + \frac{[NADH]}{Km_{NADH}} \right)}$$

(A1)

$$vMCKATC4$$

$$vMCKATC4 = \frac{sf_{vmckat}V_{max,mckat}}{\left(1 + \frac{[c4acylCoA]}{Km_{MCKAT,C4acylCoA}} + \underbrace{\frac{[AcetylCoA]}{Km_{AcetylCoA}}}_{inhibition\ by\ reaction\ product} + \sum_{n=6}^{16} \left\{ \frac{[CnKetoacylCoA]}{Km_{MCKAT,CnketoacylCoA}} + \frac{[CnAcylCoA]}{Km_{MCKAT,CnacylCoA}} \right\} \right) \left(1 + \frac{[FreeCoA]}{Km_{CoA}} + \frac{[AcetylCoA]}{Km_{AcetylCoA}} \right)}$$

(A2)

The following notations are used:

M=C4-Ketoacyl-CoAMAT

Mn=Cn-Ketoacyl-CoAMAT, n=6, 8, 10, 12, 14, 16

Hn= Cn-Hydroxyacyl-CoAMAT, n=4, 6, 8, 10, 12, 14, 16

T= total CoA pool

S = the sum of all mitochondrial CoA esters except C4-Ketoacyl-CoAMAT (M)

Nt= total of NAD+ and NADH (ND)

Acetyl =Acetyl-CoA

$vmschad = sf_{vmschad}V_{max,mschad}$

$vmckat = sf_{vmckat}V_{max,mckat}$

Then the rate equations can be rewritten as

$$vMSCHADC4 = \frac{vmschad \left(\frac{H_4(Nt-N_D)}{K_{H4}K_N} - \frac{M N_D}{K_{H4}K_N K_{eqMSCHAD}} \right)}{\left(1 + \frac{H_4}{K_{H4}} + \frac{M}{K_{Msc4}} + \frac{Q_1}{K_{Q1}} \right) \left(1 + \frac{Nt-N_D}{K_N} + \frac{N_D}{K_{N_D}} \right)} \quad (A3)$$

$$vMCKATC4 = \frac{vmckat \left(\frac{M(T-S-M)}{K_{Mc4}K_{CoA}} - \frac{Acetyl \times Acetyl}{K_{Mc4}K_{CoA} K_{eqMCKAT}} \right)}{\left(1 + \frac{M}{K_{Mc4}} + \frac{Acetyl}{K_{ACoA}} + \frac{Q_2}{K_{Q2}} \right) \left(1 + \frac{T-S-M}{K_{CoA}} + \frac{Acetyl}{K_{ACoA}} \right)} \quad (A4)$$

Where the competition part in (A3) can be expressed as

$$\frac{Q_1}{K_{Q1}} = \frac{H_6}{K_{H6}} + \frac{H_8}{K_{H8}} + \frac{H_{10}}{K_{H10}} + \frac{H_{12}}{K_{H12}} + \frac{H_{14}}{K_{H14}} + \frac{H_{16}}{K_{H16}} + \frac{M_6}{K_{M6}} + \frac{M_8}{K_{M8}} + \frac{M_{10}}{K_{M10}} + \frac{M_{12}}{K_{M12}} + \frac{M_{14}}{K_{M14}} + \frac{M_{16}}{K_{M16}}$$

Such that

$$\frac{Q_1}{K_{Q1}} = \frac{\sum_j H_j \prod_{i \neq j} K_{Hi} \prod_l K_{Mi} + \sum_l M_l \prod_j K_{Hj} \prod_{n \neq l} K_{Mn}}{\prod_j K_{Hj} \prod_l K_{Mi}}, \quad j, i, l, n \in \{6, 8, 10, 12, 14, 16\};$$

Similarly, the competition part in (A4) can be expressed as

$$\frac{Q_2}{K_{Q2}} = \frac{M_6}{K_{M4}} + \frac{M_8}{K_{M8}} + \frac{M_{10}}{K_{M10}} + \frac{M_{12}}{K_{M12}} + \frac{M_{14}}{K_{M14}} + \frac{M_{16}}{K_{M16}} + \frac{A_4}{K_{A4}} + \frac{A_6}{K_{A6}} + \frac{A_8}{K_{A8}} + \frac{A_{10}}{K_{A10}} + \frac{A_{12}}{K_{A12}} + \frac{A_{14}}{K_{A14}} + \frac{A_{16}}{K_{A16}}$$

Such that

$$\frac{Q_2}{K_{Q2}} = \frac{\sum_j M_j \prod_{i \neq j} K_{Mi} \prod_l K_{Ai} + \sum_l A_l \prod_j K_{Mj} \prod_{n \neq l} K_{An}}{\prod_j K_{Mj} \prod_l K_{Ai}}, \quad j, i \in \{6, 8, 10, 12, 14, 16\}; \quad l, n \in \{4, 6, 8, 10, 12, 14, 16\}$$

At steady state

$$VMAT \frac{dM}{dt} = 0$$

implying

$$vMSCHADC4 - vMCKATC4 = 0 \quad (A5)$$

Substituting (A3) and (A4) into (A5) we have

$$\frac{V_{mschad} \left(\frac{H4(Nt - N_D)}{K_{H4} K_N} - \frac{M N_D}{K_{H4} K_N K_{eqMSCHAD}} \right)}{\left(1 + \frac{H4}{K_{H4}} + \frac{M}{K_{Msc4}} + \frac{Q_1}{K_{Q1}} \right) \left(1 + \frac{Nt - N_D}{K_N} + \frac{N_D}{K_{N_D}} \right)} - \frac{V_{mckat} \left(\frac{M(T - S - M)}{K_{Mc4} K_{CoA}} - \frac{Acetyl \times Acetyl}{K_{Mc4} K_{CoA} K_{eqMCKAT}} \right)}{\left(1 + \frac{M}{K_{Mc4}} + \frac{Acetyl}{K_{ACoA}} + \frac{Q_2}{K_{Q2}} \right) \left(1 + \frac{T - S - M}{K_{CoA}} + \frac{Acetyl}{K_{ACoA}} \right)} = 0$$

This can be simplified to a cubic polynomial of the form

$$a M^3 + b M^2 + c M + d = 0 \quad (A6)$$

where

$$a = a_1 + a_2$$

$$b = b_1 - b_2 - b_3 + b_4$$

$$c = c_1 - c_2 + c_3 - c_4$$

$$d = d_1 + d_2$$

$$a_1 = \frac{V_{mschad} N_D}{K_{H4} K_N K_{Mc4} K_{CoA} K_{eqMSCHAD}}$$

$$b_1 = \frac{V_{mschad} H4 (Nt - N_D)}{K_{H4} K_N K_{Mc4} K_{CoA}}$$

$$b_2 = \frac{V_{mschad} N_D}{K_{H4} K_N K_{eqMSCHAD}} \times w_2$$

$$c_1 = \frac{V_{mschad} H4 (Nt - N_D)}{K_{H4} K_N} \times w_2$$

$$c_2 = \frac{V_{mschad} N_D}{K_{H4} K_N K_{eqMSCHAD}} \times w_1$$

$$d_1 = \frac{V_{mschad} H4 (Nt - N_D)}{K_{H4} K_N} \times w_1$$

$$a_2 = \frac{V_{mckat}}{K_{Mc4} K_{CoA} K_{Msc4}} \times y_2,$$

$$b_3 = \frac{V_{mckat} (T - S)}{K_{Mc4} K_{CoA} K_{eqMCKAT} K_{Msc4}} \times y_2,$$

$$b_4 = \frac{V_{mckat}}{K_{Mc4} K_{CoA}} \times y_1,$$

$$c_3 = \frac{V_{mckat} AcetylCoA \times Acetyl}{K_{Mc4} K_{CoA} K_{eqMCKAT} K_{Msc4}} \times y_2,$$

$$c_4 = \frac{V_{mckat} (T - S)}{K_{Mc4} K_{CoA}} \times y_1,$$

$$d_2 = \frac{V_{mckat} \text{Acetyl} \times \text{Acetyl}}{K_{Mc4} K_{CoA} K_{eqMCKAT}} \times y_1,$$

With

$$w_1 = 1 + \frac{T - S}{K_{CoA}} + \frac{\text{Acetyl}}{K_A} + \frac{\text{Acetyl}}{K_A} + \frac{\text{Acetyl} \times (T - S)}{K_A K_{CoA}} + \frac{\text{Acetyl} \times \text{Acetyl}}{K_A \times K_A} + \frac{Q_2}{K_{Q2}} + \frac{Q_2(T - S)}{K_{Q2} K_{CoA}} + \frac{Q_2 \times \text{Acetyl}}{K_{Q2} K_A}$$

$$w_2 = \frac{1}{K_{Mc4}} - \frac{1}{K_{CoA}} + \frac{T - S}{K_{Mc4} K_{CoA}} + \frac{\text{Acetyl}}{K_{Mc4} K_A} - \frac{\text{Acetyl}}{K_A K_{CoA}} - \frac{Q_2}{K_{Q2} K_{CoA}}$$

$$y_1 = 1 + \frac{Nt - N_D}{K_N} + \frac{N_D}{K_D} + \frac{H_4}{K_{H4}} + \frac{H_4 \times (Nt - N_D)}{K_{H4} K_N} + \frac{H_4 \times N_D}{K_{H4} K_D} + \frac{Q_1}{K_{Q1}} + \frac{Q_1(Nt - N_D)}{K_{Q1} K_N} + \frac{Q_1 \times N_D}{K_{Q1} K_D}$$

$$y_2 = 1 + \frac{Nt - N_D}{K_N} + \frac{N_D}{K_D}$$