Understanding regulation of aspartate metabolism with a model based on measured kinetic parameters

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SUPPLEMENTARY DATA

S1 Kinetic analyses and derivation of simplified rates laws

S1.1 Aspartate semi-aldehyde dehydrogenase (ASADH)

ASADH catalyses a reversible reaction and involves two substrates, AspP and NADPH, and three products, (ASA, NADP and P_i). AspP and ASA were produced enzymatically as previously described (Paris *et al.*, 2002; 2003). Results displayed in Fig. S1 were used to derive a simple equation for ASADH:

$$v_{\mathrm{ASADH}} = [\mathrm{ASADH}] \cdot (0.9[\mathrm{AspP}] - 0.23[\mathrm{ASA}]) \qquad \text{(S1)}$$

The equation is valid for the physiological values of $[P_i]$, [NADPH] and [NADP] listed in Table S1, and these concentrations are included in the numerical values.

We assumed that the rate in the forward direction (AspP $\rightarrow$ ASA) was proportional to [AspP] and that the rate in the reverse direction (ASA $\rightarrow$ AspP) was proportional to [ASA]. These assumptions were verified *a posteriori* because [ASA] and [AspP] steady-state concentrations in the model were much lower than the apparent K_m of ASADH for [ASA] and [AspP]. Rate constants for the forward and the reverse directions correspond to the apparent specificity constants (k_{cat}/K_m) for AspP and for ASA, respectively.

S1.2 Aspartate kinases (AK)

AK catalyses the phosphorylation of Asp in the presence of ATP. The products are AspP and ADP. The reaction has an equilibrium constant that strongly favours the reverse reaction (Shaw and Smith, 1977). AKs are the most complex enzymes in the Asp-derived amino acid pathway. Five isoforms exist in *Arabidopsis* (Curien *et al.*, 2005; 2007). Three are monofunctional enzymes. Among these, two are inhibited by Lys (AK2, AK3) and one (AK1) is inhibited synergistically by Lys and AdoMet. AK3 is restricted to vascular tissues (Yoshioka *et al.*, 2001) and is not taken into account in the model. The other two AK activities exist on bifunctional proteins (AKI–HSDH I and AKII–HSDH II) bearing AK and homoserine dehydrogenase (HSDH) domains. The HSDH domain catalyses the third step in the pathway leading to homoserine synthesis. AKI and AKII activities are inhibited by Thr and activated by Ala, Cys, Ile, Ser and Val (Curien *et al.*, 2005). The activators displace Thr inhibitor and activate by themselves in the absence of Thr, decreasing the K_m of the enzyme for the substrates. The activation factor and the apparent affinity for the activators are stronger for AKII than for AKI.

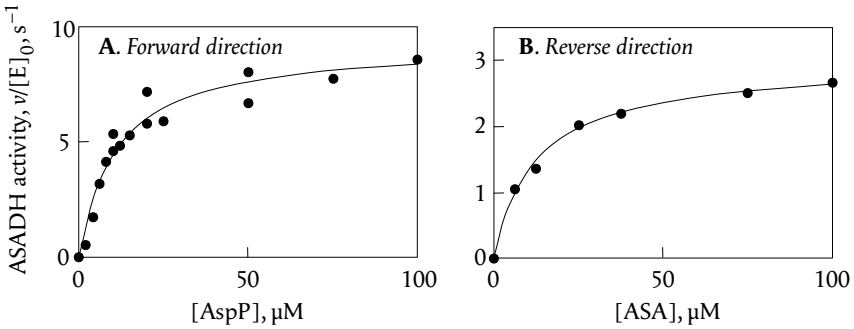

Figure S1: **Determination of ASADH kinetic properties in the forward and reverse directions. A**. ASADH activity in the forward direction was measured in Buffer A in the presence of variable amounts of AspP and 200 μM NADPH. The curve is the best fit obtained by non-linear regression analysis of the data points using the equation for a hyperbola. Apparent K_m^{AspP} = 10.6 $\pm$ 1.3 μM and apparent k_{cat} is 9.3 $\pm$ 0.3 s^{-1}. The apparent specificity constant (k_{cat}/K_m) is about 0.9 $\mu M^{-1}{\cdot}s^{-1}$. **B**. ASADH activity in the reverse direction was measured in Buffer A in the presence of 10 mM P_i, 200 μM NADP and variable [ASA]. The curve is the best fit obtained by non-linear regression analysis of the data points using the equation for a hyperbola. Apparent K_m^{ASA} = 13 $\pm$ 1 μM and apparent k_{cat} is 3.0 $\pm$ 0.1 s^{-1}. The apparent specificity constant (k_{cat}/K_m) is about 0.23 $\mu M^{-1}{\cdot}s^{-1}$.

Table S1. Near-physiological concentrations of metabolites.

Metabolite	Concentration (μM)	Metabolite	Concentration (μM)
AdoMet	20	ADP	500
Ala	400	Asp	1000
ATP	2000	Cys	15
Lys	70	NADP	200
NADPH	200	PHser	500
P_i	10000	pyruvate	1000
Ser	50	Thr	300
Val	100		

A complete mathematical description of AK kinetics is practically impossible and certainly unnecessary. Many simplifications can be made to derive simple equations that are still biologically pertinent. Rather than using mechanistic equations we favoured the derivation of empirical equations from the experimental data displayed in Figs. S2–S5, the complexity being reduced by setting [Asp], [ATP] and [ADP] to constant, near physiological values.

Rate constants for the forward direction in the absence of the allosteric inhibitors are listed in Table S2, and were obtained from Fig. S2, in which the curves are the best fits obtained by non-linear regression analysis of the experimental data points using the equation of a hyperbola.

The reversibility of the reaction was taken into account by means of the results in Fig. S3, but the activity in the reverse direction is difficult to measure directly because AspP is unstable, and an indirect procedure was used. The steady-state rate of NADPH consumption by AK plus ASADH is related to ASADH protein concentration in a hyperbolic manner, as shown in the figure. Considering that, in the absence of externally added P_i, ASADH activity in the reverse direction is negligible and assuming that AK activity in the reverse direction is proportional to [AspP], a simple mathematical analysis shows that $k_{AK}^{reverse} = k_{ASADH}^{forward} \cdot [ASADH]_{0.5}/[AK]$, where $k_{ASADH}^{forward} = 0.9 \mu M^{-1}{\cdot}s^{-1}$ (from the data in

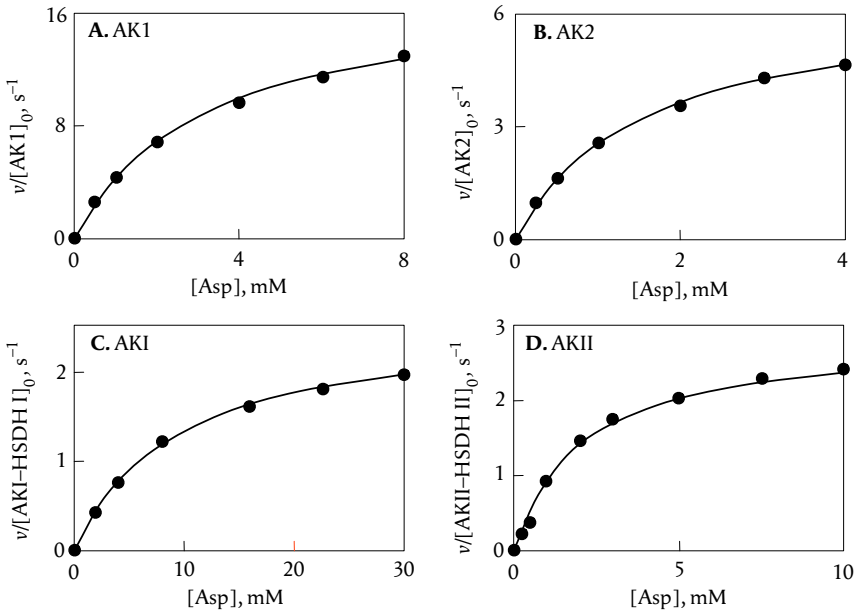

Figure S2: **Determination of AK rate constants in the forward direction as a function of [Asp] in the absence of allosteric inhibitors.** AK activities were measured in buffer A in the presence of 2 mM ATP, 500 μM ADP, 200 μM NADPH, large excess of ASADH (1 μM) and in the absence of the allosteric inhibitors. AKI and AKII activities were measured in the presence of the activators at the near-physiological concentrations of Ala, Cys, Ile, Ser and Val listed in Table S1. **A.** AK1. **B.** AK2. **C.** AKI. **D.** AKII.

Table S2. Kinetic parameters for AK isoforms from the data of Figs. S2–S3.

Parameter	AK1	AK2	AKI	AKII
Forward reaction (Fig. S2)				
k_{cat}, s^{-1}	17.7 ± 0.6	6.5 ± 0.2	2.6 ± 0.06	2.8 ± 0.12
K_m^{Asp}, μM	3080 ± 280	1560 ± 120	9200 ± 500	1980 ± 290
Reverse reaction (Fig. S3)				
Apparent rate constant, $\mu M^{-1} \cdot s^{-1}$	1.6	0.86	0.15	2.8

Fig. S1), [AK] is the AK protein concentration in the assay, $[ASADH]_{0.5}$ is the value of [ASADH] for half maximal rate of NADPH consumption, and $k_{AK}^{reverse}$ is the first-order rate constant for the transformation of AspP into Asp. From the hyperbolic curves displayed in the figure, $[ASADH]_{0.5}$ can be obtained and thus $k_{AK}^{reverse}$ can be computed. The kinetic parameters resulting from these calculations are listed in Table S2.

The responses to the allosteric effectors Lys and AdoMet were determined from the results shown in Fig. S4, which were used to derive the following equations:

$$v_{AK1} = [AK1] \cdot \frac{5.65 - 1.6[AspP]}{1 + \left[[Lys] / \left(\frac{550}{1 + [AdoMet]/3.5} \right) \right]^2} \tag{S2}$$

$$v_{AK2} = [AK2] \cdot \frac{3.15 - 0.86[AspP]}{1 + ([Lys]1 + [AdoMet]/3.5)^{1.1}} \tag{S3}$$

The numerical values in these equations are apparent values that incorporate the concentrations of ATP and ADP listed in Table S1, and [Asp] = 1.5 mM (physiological value).

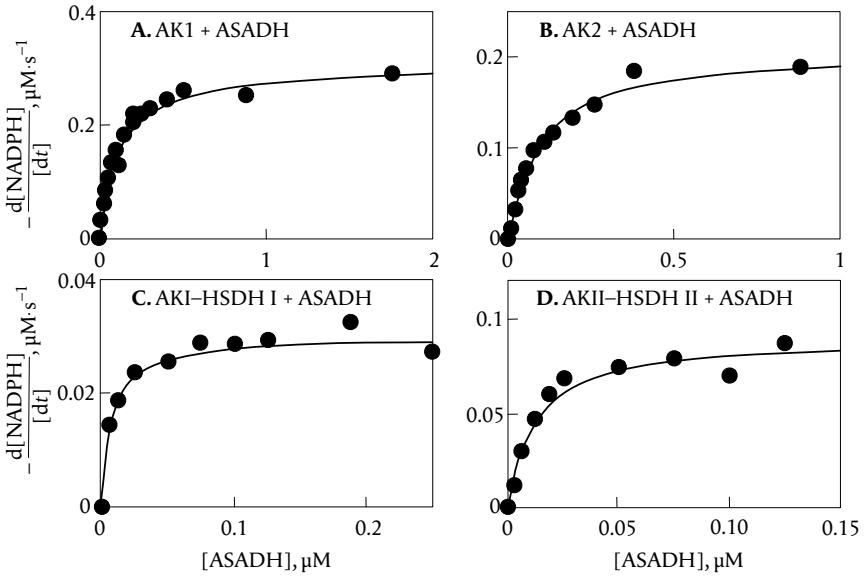

Figure S3: **Indirect determination of AK reverse rate constants.** The points are experimental. The curves correspond to simulations with the value of $k_{AK}^{reverse}$ determined with the equation $k_{AK}^{reverse} = k_{ASADH}^{forward} \cdot [ASADH]_{0.5}/[AK]$ given in the text. For AK-HSDH bifunctional enzymes, the activity of HSDH I and II were modelled according to Eqns. S6 and S7 respectively. The simulations correctly predict the dependence of NADPH consumption rate on [ASADH] in the five cases, showing that $k_{AK}^{forward}$ values were correctly estimated. **A**. AK1–ASADH with 0.06 μM AK1. $[ASADH]_{0.5}$ = 0.096 μM. Thus, $k_{AK1}^{reverse}$ = 1.6 $\mu M^{-1} \cdot s^{-1}$. **B**. AK2–ASADH with 0.1 μM AK2. $[ASADH]_{0.5}$ = 0.086 $\pm$ 0.009 μM. Thus, $k_{AK2}^{reverse}$ = 0.86 $\mu M^{-1} \cdot s^{-1}$. **C**, AKI–ASADH–HSDH I with 0.05 μM AKI–HSDH I and physiological concentrations of AKI activators (Table 1 in main paper). $[ASADH]_{0.5}$ = 0.0075 $\pm$ 0.001 μM. Thus, $k_{AKI}^{reverse}$ = 0.15 $\mu M^{-1} \cdot s^{-1}$. **D**, AKII–ASADH–HSDH II (0.05 μM AKII–HSDH II and physiological concentrations of AKII activators). $[ASADH]_{0.5}$ = 0.011 $\pm$ 0.002 μM. Thus, $k_{AKII}^{reverse}$ = 0.22 $\mu M^{-1} \cdot s^{-1}$.

The response to the allosteric effector Thr was determined from the results in Fig. S5, which were used to derive the following equations:

$$v_{AKI} = [\text{AKI-HSDH I}] \cdot \frac{0.36 - 0.15[\text{AspP}]}{(1 + [\text{Thr}]/124)^{2.6}} \tag{S4}$$

$$v_{AKII} = [\text{AKII-HSDH II}] \cdot \frac{1.35 - 0.22[\text{AspP}]}{(1 + [\text{Thr}]/109)^{2}} \tag{S5}$$

The numerical values in these equations are apparent values that incorporate the concentrations of ATP, ADP, Ala, Ser, Cys, Ile and Val listed in Table S1, and [Asp] = 1.5 mM (physiological value).

Notice that the experimentally determined parameters in Eqns. S2–S5 imply somewhat different equilibrium constants for the same reaction ($\frac{5.65}{1.6} \neq \frac{3.15}{0.86}$, etc.). These were made consistent in the model by replacing the negative coefficients in the numerators as follows: –1.6 by –1.57, –0.86 by –0.88, –0.15 by –0.10, –0.22 by –0.38. In practice the effect of these changes on the model was almost imperceptible.

These equations have several limitations. First, they do not allow changes in substrate concentrations to be simulated. Indeed, the inhibition of AKs by Lys and AdoMet is competitive, and so $K_{0.5}$ values for the allosteric effector(s) depend on substrate concentration. Much more complex equations would be required to account precisely for this complex relationship between substrate and allosteric effector(s) concentrations. As the range of

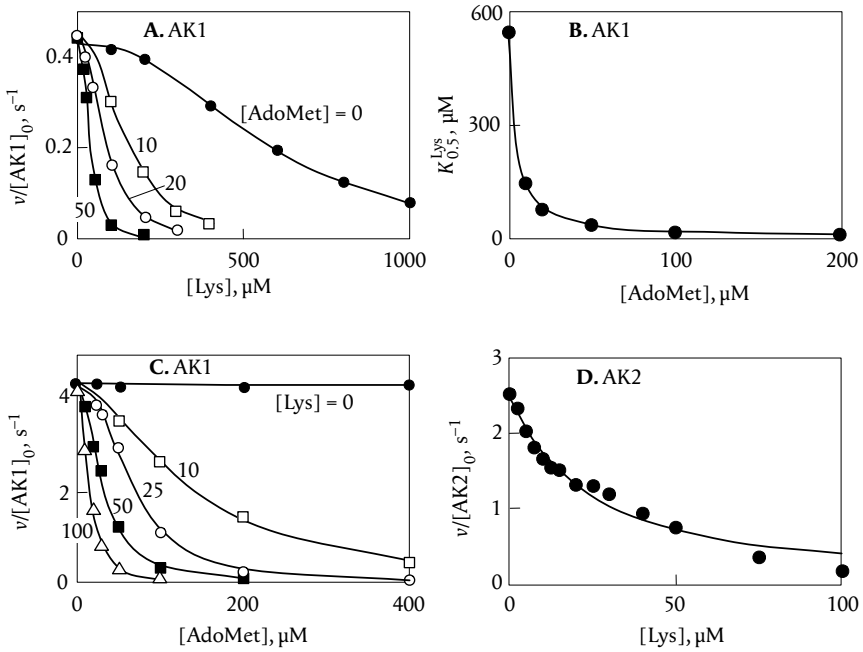

Figure S4: **Regulatory properties of monofunctional AKs.** Curves are the best fits obtained by non-linear regression analysis of the experimental data using a Hill equation. **A.** AK1 activity as a function of [Lys] for different AdoMet concentrations (from right to left [AdoMet] = 0, 10, 20, 50 μM). Values of $K_{0.5}^{\mathrm{Lys}}$ for AK1 in the presence of 0, 10, 20, 50 μM AdoMet are 552 $\pm$ 7 μM, 143 $\pm$ 5 μM, 82 $\pm$ 1.3 μM, 36 $\pm$ 3 μM respectively. The Hill coefficients were similar (about 2.3 $\pm$ 0.1). **B.** Values of $K_{0.5}^{\mathrm{Lys}}$ for AK1 calculated from the results in **A** and data points for 100 and 200 μM AdoMet that are not shown in **A**, were plotted as a function of [AdoMet]. Data points were fitted with the equation $K_{0.5}^{\mathrm{Lys}} = 550/(1 + [\mathrm{AdoMet}]/3.5)$. **C.** AK1 activity as a function of [AdoMet] for different [Lys] as indicated). **D.** AK2 activity as a function of [Lys].

[Asp] change in the chloroplast is unknown we refrained from simulating changes in [Asp]. More complex equations were not required at this step. Nevertheless, results in Fig. S2 indicate that AK rates are nearly proportional to [Asp] around the physiological range (1.5 mM) in the absence of the inhibitors. This is *a fortiori* true in their presence. In addition, $K_{0.5}$ values for the allosteric effectors are not highly sensitive to substrate concentrations (data not shown). Thus, the response to small changes in [Asp] could nevertheless be simulated with a good approximation, assuming AK rates are proportional to [Asp] and assuming $K_{0.5}$ values are independent of [Asp].

The second limitation concerns AKI and AKII. The inhibition of AKI and AKII by Thr was measured at near-physiological concentrations of the amino acid activators (Ala, Cys, Ile, Ser, Val: see Table S1). Changes in the concentration of the activators cannot be simulated with these equations. At first sight this might appear as a severe limitation, especially for Ile. Indeed, Ile is an internal variable in the model. In principle, changes in [Ile] affect AKI and AKII activities, but this is not the case with the equations used in the model. However, we have shown that the activation by Ile in the presence of Thr is quantitatively important only for values of [Ile] far above the physiological value (Curien *et al.*, 2005). Using a more complex equation for AKI and AKII that would take into account changes in [Ile] in an explicit manner is not an absolute requirement, as long as [Ile] remains close to physiological values.

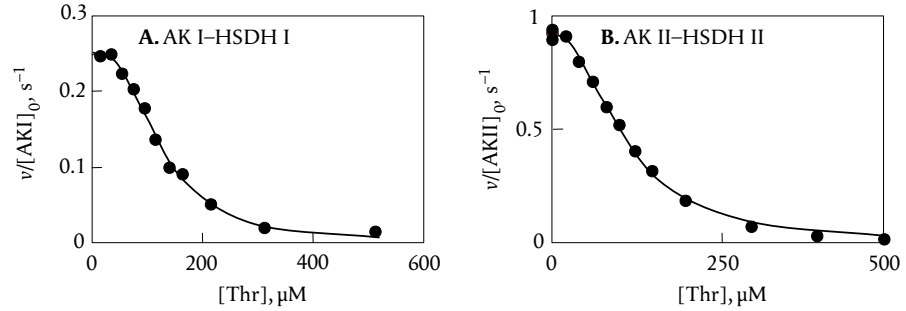

Figure S5: **Thr-sensitive AK (bifunctional AKs) regulatory properties**. AK activities of bifunctional AKI–HSDH I and AKII–HSDH II isoforms were measured in Buffer A in the presence of 1 mM Asp, 2 mM ATP and 0.5 mM ADP, 1 μM ASADH and 200 μM NADPH and in the presence of the activators at the near-physiological concentrations of Ala, Cys, Ile, Ser and Val listed in Table S1. Note that the rate of NADPH consumption by AK-HSDH plus ASADH is twice the AK rate, because each molecule of Thr transformed by AK results in oxidation of two molecules of NADPH in the two dehydrogenase reactions. Rates at steady state were divided by two to obtain the actual AK rates ($v/[E]_0$) displayed in the plots. **A**, AKI activity as a function of [Thr]. The curve is the best fit obtained by non-linear regression analysis of the experimental data points using a Hill equation: $K_{0.5}^{\mathrm{Thr}}$ = 124 $\pm$ 3 μM and h =2.6 $\pm$ 0.2 (Eqn. 4). **B**, AKII activity as a function of [Thr]. The curve is the best fit obtained by non-linear regression analysis of the experimental data points using a Hill equation: $K_{0.5}^{\mathrm{Thr}}$ = 109 $\pm$ 2 μM and h = 2.2 $\pm$ 0.1 (See Eqn. S5).

S1.3 Homoserine dehydrogenase (HSDH)

HSDH forms the second domain of the bifunctional AKI–HSDH I and AKII–HSDH II enzyme isoforms. Kinetic evidence indicates that although Thr-sensitive AK and HSDH reside on the same polypeptide they behave as independent enzymes (Curien *et al.*, 2005). We thus considered independent equations to model AK and HSDH activities. HSDH reduces ASA to Hser in the presence of NADPH. The reaction is reversible. Activity was characterized in both directions (see Fig. S6). The HSDH-catalysed reaction is much less efficient in the reverse direction than in the forward direction. In addition, the reaction is much more sensitive to Thr inhibition in the reverse direction than in the forward direction (inhibition is complete in the reverse direction and half-inhibition occurs for a concentration of Thr one order of magnitude lower).

These results led to apparent $K_{\mathrm{m}}^{\mathrm{ASA}}$ values in the forward direction of 290 $\pm$ 15 μM HSDH I and 310 $\pm$ 60 μM for HSDH II, and apparent k_{cat} values of 245 $\pm$ 6 s^{-1} and 200 $\pm$ 21 s^{-1} respectively. The apparent specificity constant ($k_{\mathrm{cat}}^{\mathrm{app}}/K_{\mathrm{m}}^{\mathrm{app}}$) for ASA is 0.84 $\mu\mathrm{M}^{-1}\cdot\,\mathrm{s}^{-1}$ for HSDH I and 0.64 for HSDH II. These values were used for the first-order rate constants of HSDH I and HSDH II in the following equations:

$$v_{\mathrm{HSDHI}} = [\mathrm{AKI\text{-}HSDHI}]\cdot 0.84\cdot\left(0.14+\frac{0.86}{1+[\mathrm{Thr}]/400}\right) \tag{S6}$$

$$v_{\mathrm{HSDHII}} = [\mathrm{AKII\text{-}HSDHII}]\cdot 0.64\cdot\left(0.25+\frac{0.75}{1+[\mathrm{Thr}]/8500}\right) \tag{S7}$$

These equations also take account of the regulation by Thr of these isoforms in the forward reaction, which was measured in buffer A in the presence of variable concentrations of Thr and the concentrations of ASA and NADPH listed in Table S1 (Fig. S6B).

A comparison of the kinetic efficiencies in the forward and the reverse directions in the presence of a physiological concentration of threonine (300 μM, Table S1) indicates that

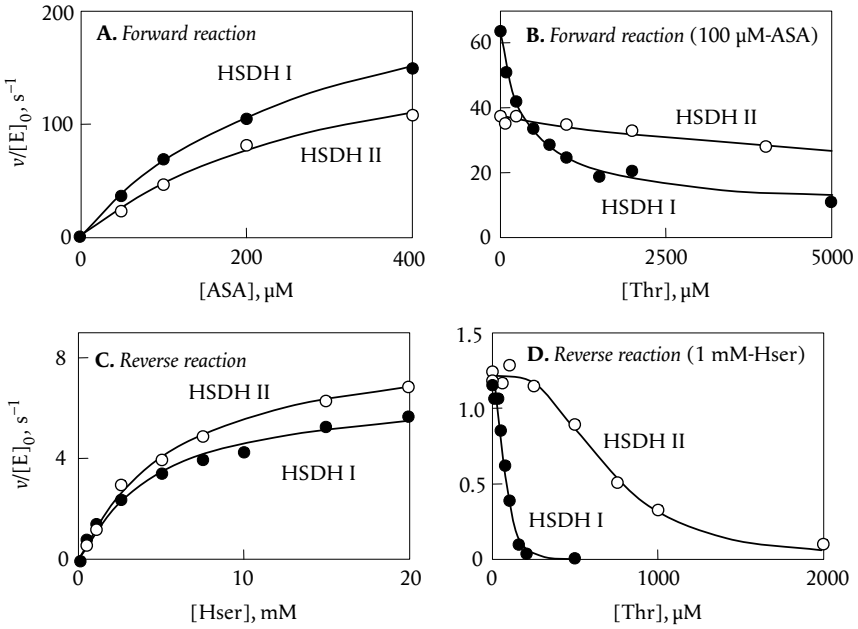

Figure S6: **Characterization of HSDH I and HSDH II kinetic and regulatory properties. A**. The activity of HSDH in the forward direction was measured in buffer A in the presence of 200 μM NADPH and variable [ASA]. The curves are the best fits obtained by non-linear regression analysis of the data points using the equation of a hyperbola. **B**. The regulatory properties of HSDH in the forward reaction were measured in buffer A in the presence of variable [Thr], with [NADPH] = 200 μM (as listed in Table S1), and [ASA] = 100 μM. Curves are best fits from non-linear regression analysis of the experimental data points using the equations of a hyperbola with a constant term at saturation by Thr, and give $K_{0.5}^{\mathrm{Thr}}$ values of 407 $\pm$ 56 μM for HSDH I and 8500 $\pm$ 1800 μM for HSDH II, and limiting residual activity of 14% for HSDH I and 25% for HSDH II. **C**. HSDH reverse reaction. HSDH activity was measured in buffer A in the presence of 200 μM NADP and variable [Hser]. Apparent $K_{\mathrm{m}}^{\mathrm{Hser}}$ were 4800 $\pm$ 600 μM and 5800 $\pm$ 500 μM for HSDH I and HSDH II, respectively. Apparent k_{cat} values were 6.8 $\pm$ 0.3 and 8.8 $\pm$ 0.3 s^{-1} for HSDH I and HSDH II, respectively. **D**. Inhibition of HSDH in the reverse direction. Activity was measured in buffer A in the presence of 200 μM NADP and 1 mM Hser. Curves are best fits from non-linear regression analysis of the experimental data points using a Hill equation: $K_{0.5}^{\mathrm{Thr}}$ = 80 $\pm$ 2 μM; h =2.9 $\pm$ 0.2 for HSDH I, and $K_{0.5}^{\mathrm{Thr}}$ = 700 $\pm$ 30, h = 2.8 $\pm$ 0.3 for HSDH II.

the apparent specificity constant of HSDH for Hser is 14 000 times lower than the apparent specificity constant for ASA. The equilibrium is thus largely displaced in the forward (physiological) direction and the reverse component of the reaction is negligible. Thus, the reaction in the reverse direction was not considered in the model. This simplification is valid as long as $[\mathrm{Hser}]_{ss}$ is lower than, or of the same order of magnitude as, the ASA substrate concentration.

We assumed that HSDHs operate far from saturation *in vivo*. The assumption could be verified *a posteriori* because $[\mathrm{ASA}]_{ss}$ in the model is much lower the apparent $K_{\mathrm{m}}^{\mathrm{ASA}}$ of HSDH. In the equations derived for HSDH I and II (Eqns. S6–S7), the rate is proportional to the enzyme concentration, to ASA substrate concentration and to a regulatory function accounting for the partial inhibition by Thr. The rate constant is the enzyme apparent specificity constant for ASA in the absence of Thr, and the concentrations of NADPH and NADP listed in Table S1 are incorporated in the numerical values.

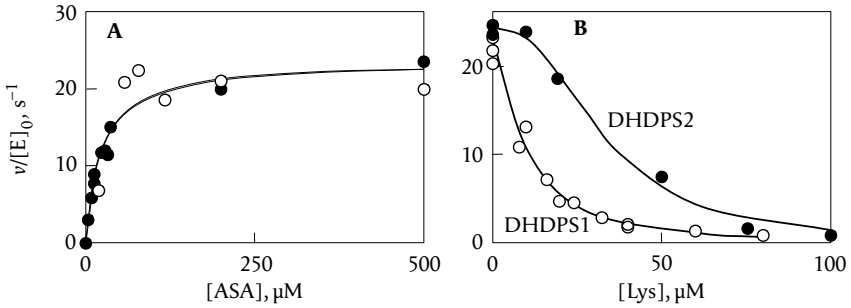

Figure S7: **Characterization of DHDPS1 and DHDPS2 kinetic and regulatory properties.** ASA substrate was prepared enzymatically (Paris *et al.*, 2003). The reaction was initiated with the enzyme (20 nM) and stopped with 5 mM Lys. The amount of ASA remaining was quantified with an excess of HSDH. The amount of ASA consumed was linear with time and enzyme concentrations (not shown). **A**. DHDPS activity was measured in Buffer A as a function of ASA concentration in the presence of 1 mM pyruvate (Table S1). K_m (20–25 μM) and k_{cat} values (20–25 s^{-1}) are similar and thus the apparent specificity constants for ASA (k_{cat}^{app}/K_m^{app}) are similar. A value of 1 $\mu M^{-1} \cdot s^{-1}$ was used for the first-order rate constant of the enzymes in Eqn. 8 and Eqn. 9. **B**. DHDPS1 and DHDPS2 activities were measured in buffer A in the presence of 500 μM ASA and 1 mM pyruvate. The curves are the best fit obtained by non-linear regression analysis of the experimental data points using a Hill equation. DHDPS1: $K_{0.5} = 10 \pm 0.8$ μM and $h = 1.6 \pm 0.2$. DHDPS2: $K_{0.5} = 33 \pm 2.5$ μM and $h = 2.5 \pm 0.3$. $K_{0.5}^{Lys}$ was independent of [ASA] in the range explored (50–500 μM).

S1.4 Dihydrodipicolinate synthase (DHDPS)

DHDPS forms dihydrodipicolinate from the irreversible condensation of pyruvate and ASA. Two enzyme isoforms (DHDPS1 and DHDPS2) with similar kinetic properties exist in *Arabidopsis* (Fig. S7). Both are strongly inhibited by Lys but have different apparent affinities for Lys. Activity was measured as a function of [ASA] and [Lys] at a constant near-physiological [pyruvate] (1 mM). Experimental results in Fig. S7 were used to derive the following simplified equations for DHDPS1 and DHDPS2, which incorporate the value [pyruvate] = 1mM:

$$v_{DHDPS1} = [DHDPS1] \cdot [ASA] \cdot \frac{1}{1 + ([Lys]/10)^2} \tag{S8}$$

$$v_{DHDPS2} = [DHDPS2] \cdot [ASA] \cdot \frac{1}{1 + ([Lys]/33)^2} \tag{S9}$$

We assumed that DHDPS operates far from saturation *in vivo*. This assumption could be verified *a posteriori* because $[ASA]_{ss}$ in the model is lower than the apparent K_m^{ASA} of DHDPS. Enzyme rate was thus assumed to be proportional to [ASA] (rate constant is the specificity constant for ASA) and to a regulatory function accounting for the inhibition by Lys. $K_{0.5}^{Lys}$ was independent of [ASA] in accordance with a catalytic inhibition (allosteric *V*-system (Curien *et al.*, 2008)).

S1.5 Homoserine kinase (HSK)

HSK phosphorylates Hser into PHser in the presence of ATP. The reaction is irreversible and is not inhibited by the products (Curien *et al.*, 2003). HSK activity was measured in the presence of variable amounts of Hser and ATP. The apparent values of K_m^{ATP} varied

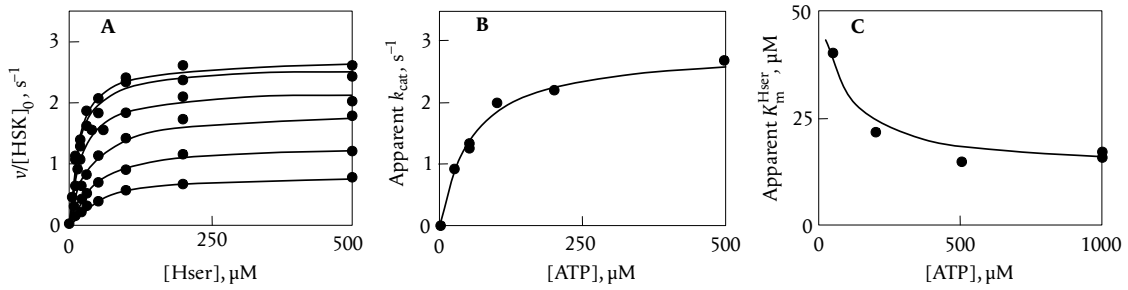

Figure S8: **HSK kinetics. A.** Activity was measured in the presence of variable [ATP] and [Hser] with a coupled assay using pyruvate kinase and lactate dehydrogenase. Curves are best fits from non-linear regression of the experimental data points to the equation for a hyperbola. **B.** Apparent k_{cat} values from **A** are plotted as a function of [ATP]. The curve is the best fit to a hyperbola from non-linear regression of the data points. **C.** The apparent K_m values from **A** are plotted as a function of [ATP].

with [Hser], and K_m^{Hser} varied with [ATP]. The results are shown in Fig. S8, and the analysis described in the legend led to following empirical equation, in which the apparent K_m^{Hser} and k_{cat} are functions of [ATP]:

$$v_{HSK} = [HSK] \cdot \frac{\left(\frac{2.85[ATP]}{54+[ATP]}\right) \cdot [Hser]}{12 + \frac{40}{1+[ATP]/80} + [Hser]} \tag{S10a}$$

For modelling purposes this was simplified by putting [ATP] = 2000 μM, i.e. 2 mM:

$$v_{HSK} = [HSK] \cdot \frac{2.8[Hser]}{14+[Hser]} \tag{S10b}$$

S1.6 Threonine synthase (TS)

TS catalyses the irreversible transformation of PHser into Thr and P_i. The enzyme operates at the branch-point between the Thr and the Met pathways in plants. The activity is inhibited by the product P_i. TS1 is allosterically activated by AdoMet. We used the complete equation for TS1 published previously (Curien *et al.*, 1998):

$$v_{TS1} = [TS1] \cdot \frac{\left(\frac{0.42+3.5[AdoMet]^2/73}{1+[AdoMet]^2/73}\right)[PHser]}{\left[\frac{250\left(\frac{1+[AdoMet]/0.5}{1+[AdoMet/1.1}\right)}{1+\frac{[AdoMet]^2}{140}}\right]\left(1+\frac{[P_i]}{1000}\right)+[PHser]} \tag{S11}$$

Note that this equation is only valid for [AdoMet] > 2 μM (Curien *et al.*, 1998).

The *Arabidopsis* genome contains a gene for a second isoform of TS (At1g72810, TS2). The cDNA for this enzyme was cloned, the enzyme overproduced in *E. coli* and purified to homogeneity. Kinetic characterization (Fig. S9) indicated that it is also activated by AdoMet but is about 120-fold less active than TS1 in the presence of near-physiological [AdoMet]. A single isoform of TS corresponding to TS1 was detected at the protein level in *Arabidopsis* leaf (Curien *et al.*, 1996). For this reason and because the

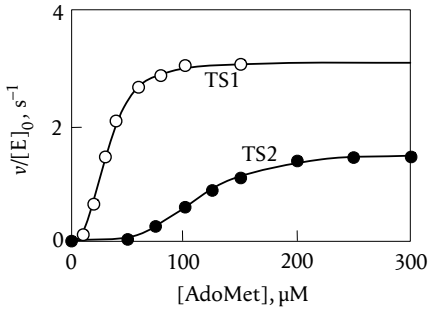

Figure S9: **Comparison of TS1 and TS2 activities.** TS activity was measured as a function of [AdoMet] in the presence of the inhibitor P_i and Phser at the near- physiological concentrations listed in Table S1, with the coupled assay described previously (Curien *et al.*, 2003). The curves are best fits to the Hill equation from non-linear regression of the experimental data. Parameters for TS1 are $K_{0.5}^{\text{AdoMet}} = 31.6 \pm 0.5$ μM, $h = 2.9 \pm 0.1$, and, for TS2, $K_{0.5}^{\text{AdoMet}} = 114 \pm 2$ μM, $h = 3.7 \pm 0.2$. At physiological [AdoMet] (20 μM), TS2 is 120-fold less active than TS1.

enzyme is much less active than TS1 at physiological [AdoMet], TS2 was not taken into account in the model.

S1.7 Cystathionine γ-synthase (CGS)

CGS catalyses the condensation of Phser and cysteine to form cystathionine and P_i. The reaction is irreversible but is inhibited by the product P_i. [Cys] and [P_i] were external variables in the model. The following equation is from Ravanel *et al.* (1998):

$$v_{\text{CGS}} = [\text{CGS}] \cdot \frac{\left(\dfrac{30}{1+460/[\text{Cys}]}\right) \cdot [\text{PHser}]}{\left(\dfrac{2500}{1+460/[\text{Cys}]}\right)\left(1+\dfrac{[\text{P}_i]}{2000}\right) + [\text{PHser}]} \tag{S12}$$

S1.8 Threonine deaminase (TD)

TD catalyses the conversion of Thr to oxobutyrate, precursor of Ile, in an irreversible reaction. TD is inhibited by Ile and this inhibition is antagonized by Val, which does not have any activating effect in the absence of Ile. The enzyme from *Arabidopsis* was previously characterized in the laboratory (Wessel *et al.*, 2000). Rather than using a complex equation based on the Monod–Wyman–Changeux model, a simple empirical equation was preferred because *in vivo* [Thr] = 300 μM $\ll K_{\text{m}}^{\text{Thr}}$. The TD rate can thus be assumed to be proportional to [Thr] (Fig. S10A). The response of the enzyme to the inhibitor Ile and the agonist Val was characterized (Fig. S10B–D). From these results, the following empirical equation, which is valid for [Thr] < 3000 μM, was derived for TD:

$$v_{\text{TD}} = [\text{TD}] \cdot \frac{0.0124[\text{Thr}]}{1+\left[[\text{Ile}]/\left(30+\dfrac{74[\text{Val}]}{610+[\text{Val}]}\right)\right]^3} \tag{S13}$$

S1.9 Lysyl-tRNA synthetase

The following equation assumes an apparent $K_{\text{m}}^{\text{Lys}}$ for the enzyme from *Canavalia ensiformis* (Fowden and Frankton, 1968), and V^{AaRS} is an adjustable parameter:

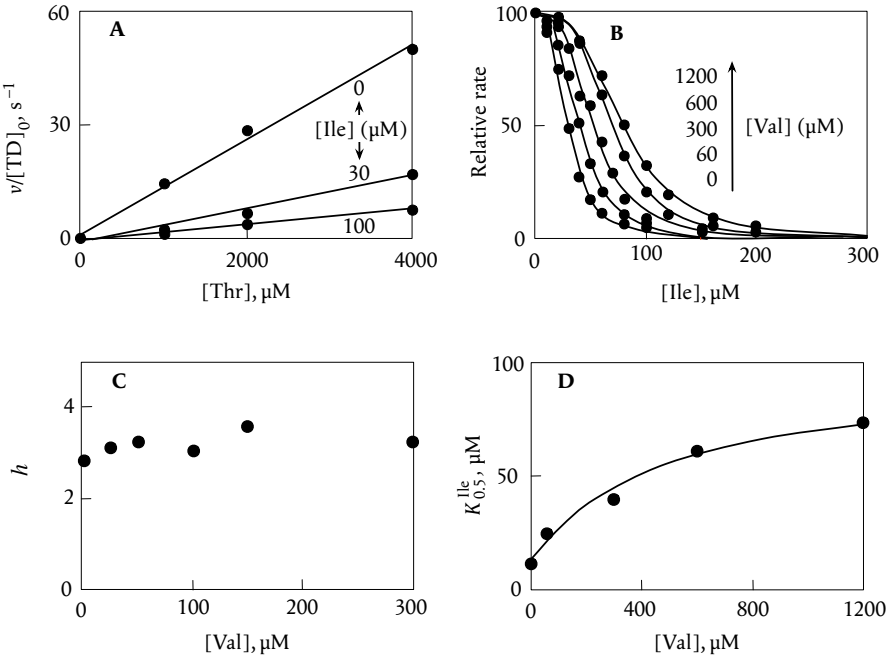

Figure S10: **Kinetic characterization of TD.** The progress of the reaction was monitored by the increase in absorbance at 230 nm due to oxobutyrate (ε = 536 mol^{-1} cm^{-1}). Activity was measured in buffer A without Hepes buffer, which was replaced by 10 mM potassium phosphate at pH 8.0 to avoid interference with the assay. **A.** TD activity was measured as a function of [Thr] in the absence or presence of Ile as indicated in the graph. In the physiological range of substrate and effector concentrations the rate is nearly proportional to substrate concentration. The slope of the line (apparent specificity constant) in the absence of Ile is 0.0124 μM$^{-1}\cdot$ s^{-1}. This value was used as the rate constant in the absence of allosteric effectors (Eqn. S13). **B.** TD activity in the presence of 1 mM-Thr was measured as a function of [Ile] for different values of [Val], and the experimental data were fitted with the Hill equation. **C.** Hill coefficients h for the curves in **B** are plotted as a function of [Val], and can be seen to be virtually independent of [Val] with a value of about 3. **D.** $K_{0.5}^{\mathrm{Ile}}$ from the curves in **B** are plotted as a function of [Val] in order to derive an empirical regulatory function for TD (see Eqn. S13).

$$v_{(\mathrm{Lys})\mathrm{tRNAsth}} = V^{\mathrm{AaRS}} \cdot \frac{[\mathrm{Lys}]}{25 + [\mathrm{Lys}]} \tag{S14}$$

S1.10 Threonyl-tRNA synthetase

The following equation assumes an $K_{\mathrm{m}}^{\mathrm{Thr}}$ for the enzyme from *Aesculus hippocastanum enzyme* (Anderson and Fowden, 1970), and V^{AaRS} is an adjustable parameter:

$$v_{(\mathrm{Thr})\mathrm{tRNAsth}} = V^{\mathrm{AaRS}} \cdot \frac{[\mathrm{Thr}]}{100 + [\mathrm{Thr}]} \tag{S15}$$

S1.11 Isoleucyl-tRNA synthetase

The following equation assumes an apparent $K_{\mathrm{m}}^{\mathrm{Lys}}$ for the enzyme from yeast (Freist and Cramer, 1983), and V^{AaRS} is an adjustable parameter:

$$v_{(\mathrm{Ile})\mathrm{tRNAsth}} = V^{\mathrm{AaRS}} \cdot \frac{[\mathrm{Ile}]}{20 + [\mathrm{Ile}]} \tag{S16}$$

S1.12 Lysine ketoglutarate reductase

The following equation assumes the K_m^{Lys} and k_{cat} given by Miron *et al.* (2000):

$$v_{LKR} = [LKR] \cdot \frac{3.1[Lys]}{13000 + [Lys]} \tag{S17}$$

S1.13 Threonine aldolase

The following equation assumes the K_m^{Thr} given by Joshi *et al.* (2006) for the enzyme from *A. thaliana*, and k_{cat} given by Liu *et al.* (1997) for the yeast enzyme:

$$v_{THA} = [THA] \cdot \frac{1.7[Thr]}{7000 + [Thr]} \tag{S18}$$

S2 Reconstitution experiments

For practical reasons, the activity of ASADH, DHDPS and HSDH could not be measured at substrate concentration levels as effectively encountered *in vivo* (μM range). In order to check whether the kinetic equations adequately accounted for the behaviour under conditions close to physiological (low substrate concentrations, enzymes working in concert), reconstitutions were carried out with purified enzymes. This was previously done for the enzymes involved in the PHser branch-point (Curien *et al.*, 2003). A similar procedure was followed with the following systems: AKII–HSDH II, ASADH, DHDPS1/2 (four-enzyme systems) and AK1, AK2, AKI–HSDH I, AKII–HSDH II, ASADH, DHDPS1, DHDPS2 (nine-enzyme system). Substrates and products (ATP, ADP, NADPH, NADP, P_i, Pyruvate) were provided at near-physiological concentrations. Enzyme concentrations were adjusted to minimize the consumption of substrates. Reconstitutions were carried out in a quartz cuvette (200 μL reaction volume). NADPH disappearance was measured at 30°C in Buffer A by monitoring the decrease in absorbance at 340 nm. Reactions were initiated by the addition of the enzyme mixture. Other details are in the Figure legends. Experimental flux values were compared to values predicted by mathematical models corresponding to the experimental conditions.

S2.1 Four-enzyme system

AKII–HSDH II, ASADH and DHDPS1 or DHDPS2 purified enzymes were mixed and supplied with Asp, ATP, ADP, NADPH, NADP and P_i as indicated in the legend to Fig. S11. We examined the influence of Lys on the kinetics of the ASA branch-point and the experimental results were compared with the prediction of a model using the corresponding equations. This preliminary investigation was done with Berkeley Madonna, but could be done with COPASI using the SBML file listed in subsection S4.3 if the appropriate enzyme concentrations are set to zero. As shown in Fig. S11, the NADPH consumption rate increased when [Lys] increased in accordance with a progressive inhibition of DHDPS and the concomitant increase in ASA steady-state concentration available to HSDH. The experiment is consistent with ASA being freely accessible to ASADH and HSDH suggesting that ASA is not channelled specifically between ASADH and HSDH. Without any parameter adjustment the curves calculated from the model adequately account for the experimental results. Thus, the simplified equations used to model the four-enzyme system are satisfactory. The difference between the model and the experiment at low [Lys] suggests that DHDPS enzymes are slightly more active or more abundant than expected (or that HSDH is less active than expected). Even here, however, the differences between the model and the experimental results are within experimental error. Note that the [Lys]

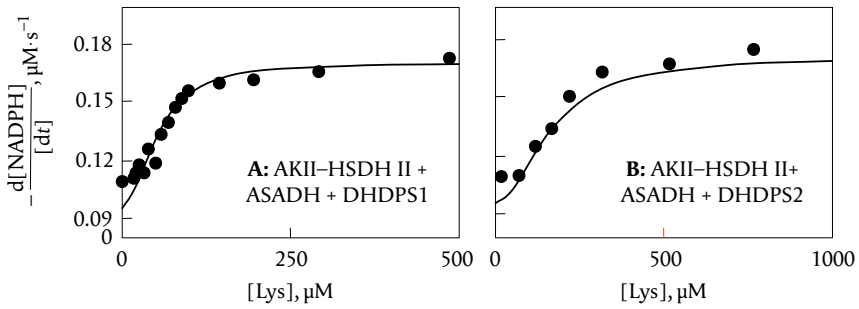

Figure S11: **Four-enzyme *in vitro* models.** The kinetic competition between HSDH and DHDPS was used to evaluate the validity of the equations derived for AK-HSDH, ASADH, DHDPS1 and DHDPS2. Quasi-steady-state rate of NADPH consumption was measured as a function of Lys in the subsystem containing AKII–HSDH II + ASADH + DHDPS1 (**A**) or AKII–HSDH II + ASADH + DHDPS2 (**B**). Experiments were carried out with 100 nM AKII–HSDHII, 1 μM ASADH and 2 μM DHDPS1 or DHDPS2 in Buffer A containing 1 mM Asp, 2 mM ATP, 500 μM ADP, 10 mM P_i, 200 μM NADPH and 200 μM NADP. Under these conditions the AK- and ASADH-catalysed steps are reversible. The reaction was initiated with addition of the enzyme mixture. The data points are experimental. The curves correspond to the simulated steady-states for the four-enzyme system with DHDPS1 or DHDPS2 when lysine concentration was varied in the same range as in the experiment. We used Eqn. S5 for AKII, Eqn. S1 for ASADH, Eqn. S7 for HSDH II and Eqn. S8 and S9 for DHDPS1 and 2, respectively. AKII forward rate constant was 0.95 s^{-1} (see Fig. S2D) because [Asp] was 1 mM in the experiment. Enzyme concentrations were set at the same value as in the experiment and [Thr] was set at 0.

required to obtain half-maximal flux in the results in Fig. S11 (70 μM with DHDPS1 and 179 μM with DHDPS2) are much higher than DHDPS1 or DHDPS2 $K_{0.5}$ values for Lys inhibition (10 and 30 μM, respectively when measured with isolated enzymes, see Fig. S7). Indeed, in the four-enzyme system, ASA steady-state concentration increased when [Lys] was increased, thus partially relieving the inhibitory effect of Lys on DHDPS. As a consequence more Lys is required for inhibition of the flux in the Lys branch. The situation is different when a Lys-sensitive AK is present in the assay (see the *in vitro* nine-enzyme system below).

S2.2 Nine-enzyme system

The activity of the nine enzymes (AK1, AK2, AKI–HSDH I, AKII–HSDH II, ASADH, DHDPS1, DHDPS2) working in concert was analysed *in vitro* as described above for the four-enzyme system. The experiment was focussed on the response to the allosteric effectors Lys, Thr and AdoMet, whose concentrations are explicit variables in the enzyme equations. These concentrations were set at the near-physiological values given in Table S1 and were varied in turn around these physiological values. To reduce the consumption of ATP, Asp and NADPH (no more than 5% consumed), monofunctional AKs and bifunctional AKs were used at 1/16th and 1/20th their cellular concentrations, respectively. ASADH and DHDPS enzymes were used at a concentration representing 1/10th their cellular concentration. Experimental results are displayed in Fig. S12 (points) and compared with the predictions of the nine-enzyme model (curves). The figure shows a good agreement between the experimental results and the predictions of the model (without any parameter adjustment), both qualitatively and quantitatively: half-decrease in flux, and, in the presence of saturating Lys, Thr or AdoMet the flux approaches a limit, as predicted.

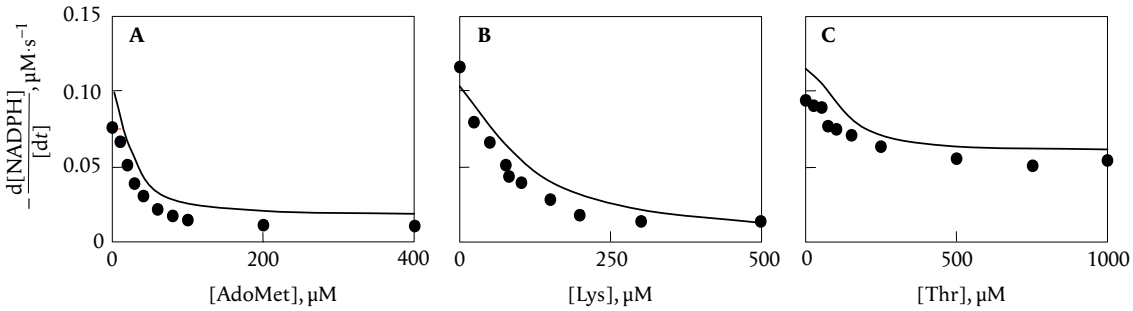

Figure S12: **Nine-enzyme *in vitro* system.** Experimental steady-state rates of NADPH consumption in the nine-enzyme system are shown as a function of [AdoMet] (**A**), [Lys] (**B**) and [Thr] (**C**) and are compared with the predictions of the model (continuous lines). When [Lys], [AdoMet] or [Thr] was varied each of the other two effectors was present at the near physiological concentrations given in Table S1; in addition Asp, ADP, pyruvate, NADPH, NADP, P_i, Ala, Ser, Cys and Val were at the concentrations given in Table S1. The reaction was carried out in buffer A and initiated by the addition of the enzyme mixture. The enzymes were AK1, AK2 (each at 0.016 μM), AKI–HSDH I and AK2–HSDH II (each at 0.03 μM), ASADH (1 μM), DHDPS1 and DHDPS2 (each at 0.15 μM). In the model corresponding to the experiments the forward rate constants for AK1, AK2, AKI and AKII were set to 4.2 s^{-1}, 2.5 s^{-1}, 0.25 s^{-1} and 0.95 s^{-1} respectively (Fig. S2). Indeed, [Asp] was set at 1 mM (rather than 1.5 mM as *in vivo*) to decrease the rate of consumption of NADPH.

The agreement indicates the good quality of the model, and also suggest the absence of complex interactions between enzymes that would affect their kinetic behaviour.

At the metabolic state of reference (Lys, Thr and AdoMet at the concentrations given in Table S1) the model of the nine-enzyme system indicates that flux of Asp conversion is about 0.075 μM·s^{-1}. This flux is about 1/20th the flux value measured *in vivo* (1.5 μM·s^{-1}) in *Lemna* cell using radioactive measurements (Giovanelli *et al.*, 1989) 1.7 μM·s^{-1} in tomato cell using NMR analyses (Rontein *et al.*, 2002). As enzyme concentrations are 1/10th or 1/20th their *in vivo* values in the assay it is reassuring to observe that flux *in vivo* is 10 to 20-fold higher than the flux *in vitro*. These results indicate that when caution is taken in the establishment of enzyme rate equations (especially when the physiological metabolic context is taken into account), *in vitro* models of complex metabolic systems can faithfully reproduce quantitative properties of real systems. This is an important point that confirms observations on a quite different system (Bakker *et al.*, 1997).

S2.3 Transient properties of the nine-enzyme *in vitro* model

Following the closure of the spectrophotometer lid (about 7 s after initiation of the reaction) the rate decreases until further decrease in absorbance is virtually linear; i.e. there is a quasi-steady-state (Fig. S13). The decrease in rate results from the slow inhibition by Lys of monofunctional AKs (Curien *et al.*, 2007). Indeed, when monofunctional AKs are absent from the assays (four-enzyme model) the rate did not decrease (not shown). In addition, the exponential decay constant obtained by curve fitting of the data points in Fig. S13 (0.013 s^{-1}) was close to the decay constant for the slow inhibition of AK obtained previously (Curien *et al.*, 2007) for AK alone (0.015 s^{-1}). The results in Fig. S13 indicate that the slow inhibition of monofunctional AKs determines the transient properties of the nine-enzyme system. The complete mathematical model described in the main text does not take into account the slow inhibition of AKs by Lys because the time constant

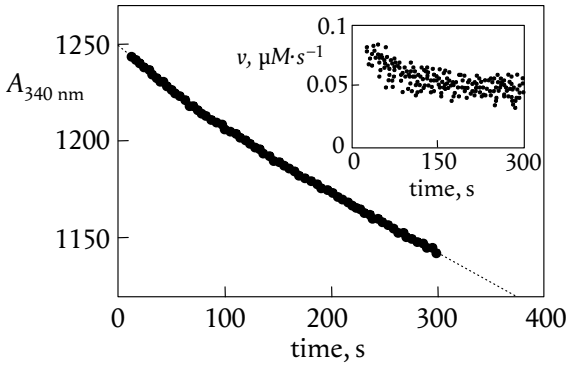

Figure S13: **Transient properties of the nine-enzyme *in vitro* model.** The progress curve shows the decrease in absorbance at 340 nm (NADPH consumption) in the nine-enzyme system in the presence of physiological concentrations of all the substrates and products. **Inset:** The rate of NADPH consumption as a function of time. A quasi-steady state is achieved after about 150 s.

of the nine-enzyme system is much smaller (about 50 s) than the time constant of the complete model described in the main text (about 400 s). When Lys-sensitive AKs are embedded in the whole 13-enzyme system they equilibrate with their ligands Lys and Lys + AdoMet before the whole system is at steady state. So no significant error results from using quasi-steady-state rate equations to describe monofunctional AK inhibition. If the half-time of the whole system were very low then the calculations of half-times in the main text would not be correct. In other words, the dynamics of the nine-enzyme system does not determine the dynamics of the Asp-derived amino acid pathway.

S3 Western blot analyses

Western blots (Fig. S14) were used to assess the co-expression of AK and DHDPS isoforms, and showed that AK1 and AK2 are co-expressed at similar levels, and that DHDPS1 and DHDPS2 are expressed at similar levels.

S4 Complete mathematical model

S4.1 Differential equations

The rate laws for the individual enzymes are detailed in Section S1 and can be assembled into the following set of differential equations:

$$\frac{d[\text{AspP}]}{dt} = v_{\text{AK1}} + v_{\text{AK2}} + v_{\text{AKI}} + v_{\text{AKII}} - v_{\text{ASADH}}$$

$$\frac{d[\text{ASA}]}{dt} = v_{\text{ASADH}} - v_{\text{DHDPS1}} - v_{\text{DHDPS2}} - v_{\text{HSDHI}} - v_{\text{HSDHII}}$$

$$\frac{d[\text{Hser}]}{dt} = v_{\text{HSDHI}} + v_{\text{HSDHII}} - v_{\text{HSK}}$$

$$\frac{d[\text{PHser}]}{dt} = v_{\text{HSK}} - v_{\text{CGS}} - v_{\text{TS1}}$$

$$\frac{d[\text{Thr}]}{dt} = v_{\text{TS1}} - v_{\text{TD}} - v_{\text{(Thr)tRNAsth}} - v_{\text{THA}}$$

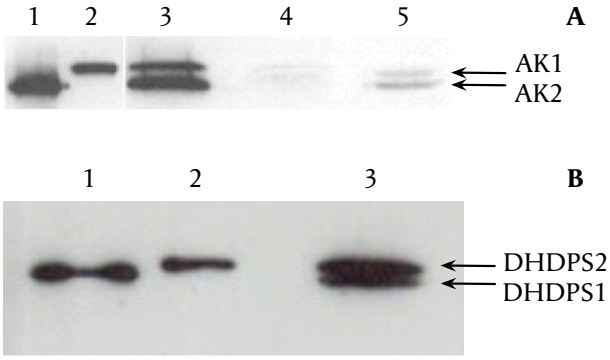

Figure S14: **Western blot analysis. A.** Proteins were separated on a 12% SDS-PAGE gel and transferred onto a nitrocellulose membrane. Western blot analyses were carried out with antibodies raised against purified AK2. **Lane 1:** purified recombinant AK2; **Lane 2:** purified AK1; **Lane 3:** AK1 + AK2; **Lane 4:** soluble proteins from the stroma of purified *Arabidopsis* chloroplasts (50 μg), showing faint bands migrating as observed for recombinant AK1 and AK2; **Lane 5:** Total soluble proteins from *Arabidopsis* cell cultures. The lower amount of Rubisco in *Arabidopsis* cell cultures allows a clear detection of the two bands, indicating co-expression of AK1 and AK2 at similar levels. **B.** Proteins were separated on a 10% SDS-PAGE gel and transferred onto a nitrocellulose membrane. Western blot analyses were carried out with antibodies raised against purified recombinant DHDPS1. **Lane 1:** purified recombinant DHDPS1; **Lane 2:** purified DHDPS2; **Lane 3:** soluble proteins from the stroma of purified *Arabidopsis* chloroplasts (50 μg), showing two bands migrating at the position corresponding to DHDPS1 (lower band) and to DHDPS2 (upper band). The experiment indicates co-expression of DHDPS1 and DHDPS2 at similar levels.

$$\frac{d[\text{Ile}]}{dt} = v_{\text{TD}} - v_{(\text{Ile})\text{tRNAsth}}$$

$$\frac{d[\text{Lys}]}{dt} = v_{\text{DHDPS1}} + v_{\text{DHDPS2}} - v_{(\text{Lys})\text{tRNAsth}} - v_{\text{THA}}$$

Explicit fixed metabolite concentrations were those of AdoMet, Cys, P_i and Val, which had the values listed in Table S1; in addition, [AK1] = [AK2] = 0.25 μM, [AKI–HSDHI] = [AKII–HSDHII] = 0.63 μM, [ASADH] = 11.6 μM, [DHDPS1] = [DHDPS2] = 1.6 μM, [HSK] = 4 μM, [CGS] = 0.7 μM, [TS1] = 7.4 μM, [TD] = 0.36 μM. [THA] = 0 unless indicated in the figure legends, [LKR] = 0 unless indicated. In addition there were implicit external variables (subsumed in the kinetic parameters), as indicated after each rate equation (see section S1).

S4.2 Complete model in Berkeley Madonna

For simulating the complete system with Berkeley Madonna it was expressed by the following script:

```
METHOD RK4

STARTTIME = 0
STOPTIME=10000
DT = 0.1
```

```
{Top model}

   {Reservoirs}
   d/dt (Aspartyl_P) = + Vak1 + Vak2 + VakI+VakII-Vasadh
      INIT Aspartyl_P =0
   d/dt (ASA) = + Vasadh - Vdhdps1 -Vdhdps2- VhsdhI -VhsdhII
      INIT ASA = 0
   d/dt (Lys) = + Vdhdps1 +Vdhdps2- VLys_tRNAS-Vlkr
      INIT Lys = 0
   d/dt (Hser) = VhsdhI + VhsdhII -Vhsk
      INIT HSer = 0
   d/dt (Phser) = + Vhsk - Vcgs - Vts1
      INIT Phser =0
   d/dt (Thr) = + Vts1 - Vtd-VThr_tRNAS-Vtha
      INIT Thr =0
   d/dt (Ile) = + Vtd-VIle_tRNAS
      INIT Ile=0

   {Flows }
Vak1 =AK1*(AK1_kforward_app_exp - AK1_kreverse_app_exp*Aspartyl_P)/
(1+(Lys/(AK1_Lys_Ki_app_exp/(1+AdoMet/ AK1_AdoMet_Ka_app_exp)))^AK1_h_exp)
Vak2 = AK2*(AK2_kforward_app_exp - AK2_kreverse_app_exp*Aspartyl_P)/
(1+(Lys/ AK2_Lys_Ki_app_exp)^AK2_h_exp)
VakI =AKHSDHI*(AKI_kforward_app_exp - AKI_kreverse_app_exp*Aspartyl_P)*1/
(1+(Thr/ AKI_Thr_Ki_app_exp)^AKI_h_exp)
 VakII =AKHSDHII*(AKII_kforward_app_exp - AKII_kreverse_app_exp*Aspartyl_P)/
(1+(Thr/ AKII_Thr_Ki_app_exp)^AKII_h_exp)
 Vasadh=ASADH*(ASADH_kforward_app_exp*Aspartyl_P- ASADH_kreverse_app_exp*ASA)
 Vdhdps1= DHDPS1* DHDPS1_k_app_exp *ASA*(1/(1+(Lys/
DHDPS1_Lys_Ki_app_exp)^ DHDPS1_h_exp))
Vdhdps2=DHDPS2* DHDPS2_k_app_exp *ASA*(1/(1+(Lys/ DHDPS2_Lys_Ki_app_exp)^ DHDPS2_h_exp))
 VhsdhI=AKHSDHI* HSDHI_kforward_app_exp *ASA*( HSDHI_Thr_relative_residual_activity_app_exp
+ HSDHI_Thr_relative_inhibition_app_exp /(1+Thr/ HSDHI_Thr_Ki_app_exp))
VhsdhII=AKHSDHII* HSDHII_kforward_app_exp *ASA*( HSDHII_Thr_relative_residual_activity_app_exp
+ HSDHII_Thr_relative_inhibition_app_exp /(1+Thr/ HSDHII_Thr_Ki_app_exp))
Vhsk=HSK* HSK_kcat_app_exp *Hser/( HSK_Hser_app_exp +Hser)
Vts1=TS1*(TS1_kcatmin_exp + TS1_AdoMet_kcatmax_exp *AdoMet^TS1_h_exp /
TS1_AdoMet_Ka1_exp)/(1+AdoMet^TS1_h_exp / TS1_AdoMet_Ka1_exp)*Phser/((1+Phosphate/
 TS1_Phosphate_Ki_exp)*( TS1_AdoMEt_Km_no_AdoMet_exp *(1+AdoMet/ TS1_AdoMet_Ka2_exp)/
(1+AdoMet/ TS1_AdoMet_Ka3_exp))/(1+AdoMet^TS1_h_exp / TS1_AdoMet_Ka4_exp)+Phser)
Vcgs= CGS*( CGS_kcat_exp /(1+ CGS_Cys_Km_exp /Cys))*Phser/
(( CGS_Phser_Km_exp /(1+ CGS_Cys_Km_exp /Cys))*(1+Phosphate/ CGS_Phosphate_Ki_exp)+ Phser)
 Vtd = TD*TD_k_app_exp*Thr/(1+(Ile/( TD_Ile_Ki_no_Val_app_exp + TD_Val_Ka1_app_exp *Val/
( TD_Val_Ka2_app_exp +Val)))^ TD_h_app_exp)
VLys_tRNAS = Lys_tRNAS_Vmax *Lys/(Lys_tRNAS_Lys_Km+Lys)
VThr_tRNAS = Thr_tRNAS_Vmax *Thr/(Thr_tRNAS_Thr_Km+Thr)
VIle_tRNAS =Ile_tRNAS_Vmax *Ile/(Ile_tRNAS_Ile_Km+Ile)
Vtha= THA*THA_kcat_exp*Thr/(THA_Thr_Km_exp+Thr)
Vlkr= LKR*LKR_kcat_exp*Lys/(LKR_Lys_Km_exp+Lys)


   {Functions}
 AK1=0.25
 AK2 = 0.25
 AKHSDHII= 0.63
 AKHSDHI = 0.63
 ASADH=11.6
 DHDPS1 = 1.6
 DHDPS2=1.6
 HSK = 4
 TS1 = 7.4
 TD=0.36
 CGS = 0.7
 THA=0
 LKR=0
 AdoMet=20
```

```
 Cys=15
 Phosphate=10000
Val=100
AK1_kforward_app_exp=5.65
AK1_kreverse_app_exp=1.57
AK1_Lys_Ki_app_exp=550
AK1_AdoMet_Ka_app_exp=3.5
AK1_h_exp=2
AK2_kforward_app_exp=3.15
AK2_kreverse_app_exp=0.88
AK2_Lys_Ki_app_exp=22
AK2_h_exp=1.1
AKI_kforward_app_exp=0.36
AKI_kreverse_app_exp=0.10
AKI_Thr_Ki_app_exp=124
AKI_h_exp=2
AKII_kforward_app_exp=1.35
AKII_kreverse_app_exp=0.38
AKII_Thr_Ki_app_exp=109
AKII_h_exp=2
ASADH_kforward_app_exp=0.9
ASADH_kreverse_app_exp=0.23
DHDPS1_k_app_exp=1
DHDPS1_Lys_Ki_app_exp=10
DHDPS1_h_exp=2
DHDPS2_k_app_exp=1
DHDPS2_Lys_Ki_app_exp=33
DHDPS2_h_exp=2
HSDHI_kforward_app_exp=0.84
HSDHI_Thr_relative_residual_activity_app_exp=0.15
HSDHI_Thr_relative_inhibition_app_exp=0.85
HSDHI_Thr_Ki_app_exp=400
HSDHII_kforward_app_exp=0.64
HSDHII_Thr_relative_residual_activity_app_exp=0.25
HSDHII_Thr_relative_inhibition_app_exp=0.75
HSDHII_Thr_Ki_app_exp=8500
HSK_kcat_app_exp=2.8
HSK_Hser_app_exp=14
TS1_kcatmin_exp=0.42
TS1_AdoMet_kcatmax_exp=3.5
TS1_AdoMEt_Km_no_AdoMet_exp=250
TS1_AdoMet_Ka1_exp=73
TS1_AdoMet_Ka2_exp=0.5
TS1_AdoMet_Ka3_exp=1.09
TS1_AdoMet_Ka4_exp=142
TS1_Phosphate_Ki_exp=1000
TS1_h_exp=2
CGS_kcat_exp=30
CGS_Cys_Km_exp=460
CGS_Phser_Km_exp=2500
CGS_Phosphate_Ki_exp=2000
TD_k_app_exp=0.0124
TD_Ile_Ki_no_Val_app_exp=30
TD_Val_Ka1_app_exp=73
TD_Val_Ka2_app_exp=615
TD_h_app_exp=3
Lys_tRNAS_Vmax=0.43
Lys_tRNAS_Lys_Km=25
Thr_tRNAS_Vmax=0.43
Thr_tRNAS_Thr_Km=100
Ile_tRNAS_Vmax=0.43
Ile_tRNAS_Ile_Km=20
THA_kcat_exp=1.7
THA_Thr_Km_exp=7100
LKR_kcat_exp=3.1
LKR_Lys_Km_exp=13000
```

S4.3 Complete model in SBML

For simulating the complete system with COPASI it was expressed in SBML as follows:

```
<?xml version="1.0" encoding="UTF-8"?>
<sbml level="2" version="3" xmlns="http://www.sbml.org/sbml/level2/version3">
<model  name="AspPathway">
<listOfUnitDefinitions>
 <unitDefinition id="per_time">
 <listOfUnits>
 <unit kind="second" exponent="-1"/>
 </listOfUnits>
 </unitDefinition>
 <unitDefinition id="per_litre">
 <listOfUnits>
 <unit kind="litre" exponent="-1"/>
 </listOfUnits>
 </unitDefinition>
 <unitDefinition id="mole_per_litre">
 <listOfUnits>
 <unit kind="mole" exponent="1"/>
 <unit kind="litre" exponent="-1"/>
 </listOfUnits>
 </unitDefinition>
 <unitDefinition id="mole2_per_litre2">
 <listOfUnits>
 <unit kind="mole" exponent="2"/>
 <unit kind="litre" exponent="-2"/>
 </listOfUnits>
 </unitDefinition>
 <unitDefinition id="litre_per_mole_per_time">
 <listOfUnits>
 <unit kind="litre" exponent="1"/>
 <unit kind="mole" exponent="-1"/>
 <unit kind="second" exponent="-1"/>
 </listOfUnits>
 </unitDefinition>
 <unitDefinition id="mole_per_time">
 <listOfUnits>
 <unit kind="mole" exponent="1"/>
 <unit kind="second" exponent="-1"/>
 </listOfUnits>
 </unitDefinition>
 </listOfUnitDefinitions>
 <listOfCompartments>
 <compartment id="c1" size="1"/>
 </listOfCompartments>
 <listOfSpecies>
 <species id="Asp" compartment="c1" initialConcentration="1500.0"/>
 <species id="AK1" compartment="c1" initialConcentration="0.25"/>
 <species id="Lys" compartment="c1" initialConcentration="0.0"/>
 <species id="AdoMet" compartment="c1" initialConcentration="20.0"/>
 <species id="Aspartyl_P" compartment="c1" initialConcentration="0"/>
 <species id="AK2" compartment="c1" initialConcentration="0.25"/>
 <species id="AKHSDHI" compartment="c1" initialConcentration="0.63"/>
 <species id="AKHSDHII" compartment="c1" initialConcentration="0.63"/>
 <species id="Thr" compartment="c1" initialConcentration="0.0"/>
 <species id="ASADH" compartment="c1" initialConcentration="11.6"/>
 <species id="ASA" compartment="c1" initialConcentration="0.0"/>
 <species id="DHDPS1" compartment="c1" initialConcentration="1.6"/>
 <species id="DHDPS2" compartment="c1" initialConcentration="1.6"/>
 <species id="Hser" compartment="c1" initialConcentration="0.0"/>
 <species id="PHser" compartment="c1" initialConcentration="0.0"/>
 <species id="HSK" compartment="c1" initialConcentration="4.0"/>
 <species id="TS1" compartment="c1" initialConcentration="7.4"/>
 <species id="Phosphate" compartment="c1" initialConcentration="10000"/>
```

```
<species id="Cys" compartment="c1" initialConcentration="15"/>
<species id="CGS" compartment="c1" initialConcentration="0.7"/>
<species id="Cysta" compartment="c1" initialConcentration="0.0"/>
<species id="TD" compartment="c1" initialConcentration="0.36"/>
<species id="Ile" compartment="c1" initialConcentration="0.0"/>
<species id="Val" compartment="c1" initialConcentration="100.0"/>
</listOfSpecies>
<listOfReactions>
<reaction id="Vak1" reversible="false">
<listOfReactants>
<speciesReference species="Asp"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="Aspartyl_P"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="AK1"/>
<modifierSpeciesReference species="Lys"/>
<modifierSpeciesReference species="AdoMet"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply> <times/> <ci>c1</ci> <ci> AK1 </ci>
  <apply>
    <divide/>
    <apply>
      <minus/> <ci> AK1_kforward_app_exp </ci>
      <apply>
        <times/> <ci> AK1_kreverse_app_exp </ci> <ci> Aspartyl_P </ci>
      </apply>
    </apply>
    <apply>
      <plus/> <cn>1</cn>
      <apply>
        <power/>
        <apply>
          <divide/>
          <apply>
            <divide/> <ci>Lys</ci> <ci>AK1_Lys_Ki_app_exp</ci>
          </apply>
          <apply>
            <plus/> <cn>1</cn>
            <apply>
              <divide/> <ci>AdoMet</ci> <ci>AK1_AdoMet_Ka_app_exp</ci>
            </apply>
          </apply>
        </apply>
        <ci>AK1_nH_exp</ci>
      </apply>
    </apply>
  </apply>
</apply>
</math>
<listOfParameters>
<parameter id="AK1_kforward_app_exp" value="5.65" units="per_time"/>
<parameter id="AK1_kreverse_app_exp" value="1.57"
                          units="litre_per_mole_per_time"/>
<parameter id="AK1_Lys_Ki_app_exp" value="550" units="mole_per_litre"/>
<parameter id="AK1_AdoMet_Ka_app_exp" value="3.5" units="mole_per_litre"/>
<parameter id="AK1_nH_exp" value="2" units="dimensionless"/>
</listOfParameters>
</kineticLaw>
</reaction>

<reaction id="Vak2" reversible="false">
<listOfReactants>
```

```
<speciesReference species="Asp"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="Aspartyl_P"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="AK2"/>
<modifierSpeciesReference species="Lys"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
  <times/> <ci>c1</ci> <ci> AK2 </ci>
  <apply>
    <divide/>
    <apply>
      <minus/> <ci> AK2_kforward_app_exp  </ci>
      <apply>
        <times/> <ci> AK2_kreverse_app_exp </ci> <ci> Aspartyl_P </ci>
      </apply>
    </apply>
    <apply>
      <plus/> <cn>1</cn>
      <apply>
        <power/>
        <apply>
          <divide/> <ci>Lys</ci> <ci>AK2_Lys_Ki_app_exp</ci>
        </apply>
        <ci>AK2_nH_exp</ci>
      </apply>
    </apply>
  </apply>
</apply>
</math>
<listOfParameters>
<parameter id="AK2_kforward_app_exp" value="3.15" units="per_time"/>
<parameter id="AK2_kreverse_app_exp" value="0.88"
                               units="litre_per_mole_per_time"/>
<parameter id="AK2_Lys_Ki_app_exp" value="22" units="mole_per_litre"/>
<parameter id="AK2_nH_exp" value="1.1" units="dimensionless"/>
</listOfParameters>
</kineticLaw>
</reaction>
<reaction id="VakI" reversible="false">
<listOfReactants>
<speciesReference species="Asp"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="Aspartyl_P"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="AKHSDHI"/>
<modifierSpeciesReference species="Thr"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
  <times/> <ci>c1</ci> <ci> AKHSDHI </ci>
  <apply>
    <divide/>
    <apply>
      <minus/> <ci> AKI_kforward_app_exp </ci>
      <apply>
        <times/> <ci> AKI_kreverse_app_exp </ci> <ci> Aspartyl_P </ci>
      </apply>
    </apply>
```

```
    <apply>
      <plus/> <cn>1</cn>
      <apply>
        <power/>
        <apply>
          <divide/> <ci>Thr</ci> <ci>AKI_Thr_Ki_app_exp</ci>
        </apply>
        <ci>AKI_nH_exp</ci>
      </apply>
    </apply>
  </apply>
</apply>
</math>
<listOfParameters>
<parameter id="AKI_kforward_app_exp" value="0.36" units="per_time"/>
<parameter id="AKI_kreverse_app_exp" value="0.10"
                           units="litre_per_mole_per_time"/>
<parameter id="AKI_Thr_Ki_app_exp" value="124" units="mole_per_litre"/>
<parameter id="AKI_nH_exp" value="2" units="dimensionless"/>
</listOfParameters>
</kineticLaw>
</reaction>
 <reaction id="VakII" reversible="false">
<listOfReactants>
<speciesReference species="Asp"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="Aspartyl_P"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="AKHSDHII"/>
<modifierSpeciesReference species="Thr"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
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  <apply>
    <divide/>
    <apply>
      <minus/> <ci> AKII_kforward_app_exp </ci>
      <apply>
        <times/> <ci> AKII_kreverse_app_exp </ci> <ci> Aspartyl_P </ci>
      </apply>
    </apply>
    <apply>
      <plus/> <cn>1</cn>
      <apply>
        <power/>
        <apply>
          <divide/> <ci>Thr</ci> <ci>AKII_Thr_Ki_app_exp</ci>
        </apply>
        <ci>AKII_nH_exp</ci>
      </apply>
    </apply>
  </apply>
</apply>
</math>
<listOfParameters>
<parameter id="AKII_kforward_app_exp" value="1.35" units="per_time"/>
<parameter id="AKII_kreverse_app_exp" value="0.38"
                             units="litre_per_mole_per_time"/>
<parameter id="AKII_Thr_Ki_app_exp" value="109" units="mole_per_litre"/>
<parameter id="AKII_nH_exp" value="2" units="dimensionless"/>
</listOfParameters>
</kineticLaw>
```

```
</reaction>
<reaction id="Vasadh" reversible="false">
<listOfReactants>
<speciesReference species="Aspartyl_P"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="ASA"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="ASADH"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
  <times/> <ci>c1</ci> <ci> ASADH </ci>
  <apply>
    <minus/>
    <apply>
      <times/> <ci> ASADH_kforward_app_exp </ci> <ci> Aspartyl_P </ci>
    </apply>
    <apply>
      <times/> <ci> ASADH_kreverse_app_exp </ci> <ci> ASA </ci>
    </apply>
  </apply>
</apply>
</math>
<listOfParameters>
<parameter id="ASADH_kforward_app_exp" value="0.9"
                          units="litre_per_mole_per_time"/>
<parameter id="ASADH_kreverse_app_exp" value="0.23"
                          units="litre_per_mole_per_time"/>
</listOfParameters>
</kineticLaw>
</reaction>
<reaction id="Vdhdps1" reversible="false">
<listOfReactants>
<speciesReference species="ASA"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="Lys"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="DHDPS1"/>
<modifierSpeciesReference species="Lys"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
  <times/> <ci>c1</ci> <ci> DHDPS1_k_app_exp </ci>
                          <ci> DHDPS1 </ci> <ci> ASA </ci>
  <apply>
    <divide/>
    <cn> 1 </cn>
    <apply>
      <plus/> <cn> 1 </cn>
      <apply>
        <power/>
        <apply>
          <divide/> <ci> Lys </ci> <ci> DHDPS1_Lys_Ki_app_exp </ci>
        </apply>
        <ci> DHDPS1_nH_exp </ci>
      </apply>
    </apply>
  </apply>
</apply>
</math>
```

```
<listOfParameters>
<parameter id="DHDPS1_k_app_exp" value="1" units="mole_per_litre"/>
<parameter id="DHDPS1_Lys_Ki_app_exp" value="10" units="mole_per_litre"/>
<parameter id="DHDPS1_nH_exp" value="2" units="dimensionless"/>
</listOfParameters>
</kineticLaw>
</reaction>
<reaction id="Vdhdps2" reversible="false">
<listOfReactants>
<speciesReference species="ASA"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="Lys"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="DHDPS2"/>
<modifierSpeciesReference species="Lys"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
  <times/> <ci>c1</ci> <ci> DHDPS2_k_app_exp </ci>
                          <ci> DHDPS2 </ci> <ci> ASA </ci>
  <apply>
    <divide/>
    <cn> 1 </cn>
    <apply>
      <plus/> <cn> 1 </cn>
      <apply>
        <power/>
        <apply>
          <divide/> <ci> Lys </ci> <ci> DHDPS2_Lys_Ki_app_exp </ci>
        </apply>
        <ci> DHDPS2_nH_exp </ci>
      </apply>
    </apply>
  </apply>
</apply>
</math>
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<parameter id="DHDPS2_k_app_exp" value="1" units="mole_per_litre"/>
<parameter id="DHDPS2_Lys_Ki_app_exp" value="33" units="mole_per_litre"/>
<parameter id="DHDPS2_nH_exp" value="2" units="dimensionless"/>
</listOfParameters>
</kineticLaw>
</reaction>

<reaction id="Vhsdh1" reversible="false">
<listOfReactants>
<speciesReference species="ASA"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="Hser"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="AKHSDHI"/>
<modifierSpeciesReference species="Thr"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
  <times/> <ci>c1</ci> <ci> HSDHI_kforward_app_exp </ci>
                       <ci> AKHSDHI </ci> <ci> ASA </ci>
  <apply>
    <plus/> <ci> HSDHI_Thr_relative_residual_activity_app_exp </ci>
    <apply>
```

```
      <divide/> <ci> HSDHI_Thr_relative_inhibition_app_exp </ci>
      <apply>
        <plus/> <cn> 1 </cn>
        <apply>
          <divide/> <ci> Thr </ci> <ci> HSDHI_Thr_Ki_app_exp </ci>
        </apply>
      </apply>
    </apply>
  </apply>
</apply>
</math>
<listOfParameters>
<parameter id="HSDHI_kforward_app_exp" value="0.84"
                          units="litre_per_mole_per_time"/>
<parameter id="HSDHI_Thr_relative_residual_activity_app_exp"
                          value="0.14" units="dimensionless"/>
<parameter id="HSDHI_Thr_relative_inhibition_app_exp"
                          value="0.86" units="dimensionless"/>
<parameter id="HSDHI_Thr_Ki_app_exp" value="400" units="mole_per_litre"/>
</listOfParameters>
</kineticLaw>
</reaction>

<reaction id="Vhsdh2" reversible="false">
<listOfReactants>
<speciesReference species="ASA"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="Hser"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="AKHSDHII"/>
<modifierSpeciesReference species="Thr"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
  <times/> <ci>c1</ci> <ci> HSDHII_kforward_app_exp </ci>
                          <ci> AKHSDHII </ci> <ci> ASA </ci>
  <apply>
    <plus/> <ci> HSDHII_Thr_relative_residual_activity_app_exp </ci>
    <apply>
      <divide/> <ci> HSDHII_Thr_relative_inhibition_app_exp </ci>
      <apply>
        <plus/> <cn> 1 </cn>
        <apply>
          <divide/> <ci> Thr </ci> <ci> HSDHII_Thr_Ki_app_exp </ci>
        </apply>
      </apply>
    </apply>
  </apply>
</apply>
</math>
<listOfParameters>
<parameter id="HSDHII_kforward_app_exp" value="0.64"
                          units="litre_per_mole_per_time"/>
<parameter id="HSDHII_Thr_relative_residual_activity_app_exp" value="0.25"
                          units="dimensionless"/>
<parameter id="HSDHII_Thr_relative_inhibition_app_exp" value="0.75"
                          units="dimensionless"/>
<parameter id="HSDHII_Thr_Ki_app_exp" value="8500"
                          units="mole_per_litre"/>
</listOfParameters>
</kineticLaw>
</reaction>
```

```
<reaction id="Vhsk" reversible="false">
<listOfReactants>
<speciesReference species="Hser"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="PHser"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="HSK"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
  <divide/>
  <apply>
    <times/> <ci>c1</ci> <ci> HSK_kcat_app_exp  </ci>
                    <ci> HSK </ci> <ci> Hser </ci>
  </apply>
  <apply>
    <plus/> <ci> HSK_Hser_app_exp </ci> <ci> Hser </ci>
  </apply>
</apply>
</math>
<listOfParameters>
<parameter id="HSK_kcat_app_exp" value="2.8" units="per_time"/>
<parameter id="HSK_Hser_app_exp" value="14" units="mole_per_litre"/>
</listOfParameters>
</kineticLaw>
</reaction>

<reaction id="Vts1" reversible="false">
<listOfReactants>
<speciesReference species="PHser"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="Thr"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="TS1"/>
<modifierSpeciesReference species="Phosphate"/>
<modifierSpeciesReference species="AdoMet"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
  <times/> <ci>c1</ci> <ci> TS1 </ci> <ci> PHser </ci>
  <apply>
    <divide/>
    <apply>
      <divide/>
      <apply>
        <plus/>
        <ci>TS1_kcatmin_exp</ci>
        <apply>
          <times/>
          <ci>TS1_AdoMet_kcatmax_exp </ci>
          <apply>
            <divide/>
            <apply>
              <power/>
              <ci>AdoMet</ci>
              <ci>TS1_nH_exp</ci>
             </apply>
             <ci>TS1_AdoMet_Ka1_exp</ci>
            </apply>
          </apply>
```

```
    </apply>
  <apply>
    <plus/>
    <cn>1</cn>
    <apply>
      <divide/>
      <apply>
        <power/>
        <ci>AdoMet</ci>
        <ci>TS1_nH_exp</ci>
      </apply>
      <ci>TS1_AdoMet_Ka1_exp</ci>
    </apply>
  </apply>
</apply>
<apply>
  <plus/>
  <apply>
    <times/>
    <ci>TS1_AdoMEt_Km_no_AdoMet_exp </ci>
    <apply>
      <divide/>
      <apply>
        <divide/>
        <apply>
          <plus/>
          <cn>1</cn>
          <apply>
            <divide/>
            <ci>AdoMet</ci>
            <ci>TS1_AdoMet_Ka2_exp</ci>
          </apply>
        </apply>
        <apply>
          <plus/>
          <cn>1</cn>
          <apply>
            <divide/>
            <ci>AdoMet</ci>
            <ci>TS1_AdoMet_Ka3_exp</ci>
          </apply>
        </apply>
      </apply>
      <apply>
        <plus/>
        <cn>1</cn>
        <apply>
          <divide/>
          <apply>
            <power/>
            <ci>AdoMet</ci>
            <ci>TS1_nH_exp </ci>
          </apply>
          <ci>TS1_AdoMet_Ka4_exp</ci>
        </apply>
      </apply>
    </apply>
    <apply>
      <plus/>
      <cn>1</cn>
      <apply>
        <divide/>
        <ci>Phosphate</ci>
        <ci>TS1_Phosphate_Ki_exp</ci>
      </apply>
    </apply>
```

```
      </apply>
      <ci>PHser</ci>
    </apply>
  </apply>
</apply>
</math>
<listOfParameters>
<parameter id="TS1_kcatmin_exp" value="0.42" units="dimensionless"/>
<parameter id="TS1_AdoMet_kcatmax_exp" value="3.5" units="dimensionless"/>
<parameter id="TS1_AdoMet_Ka1_exp" value="73" units="mole2_per_litre2"/>
<parameter id="TS1_nH_exp" value="2" units="dimensionless"/>
<parameter id="TS1_Phosphate_Ki_exp" value="1000" units="mole_per_litre"/>
<parameter id="TS1_AdoMEt_Km_no_AdoMet_exp" value="250" units="dimensionless"/>
<parameter id="TS1_AdoMet_Ka2_exp" value="0.5" units="dimensionless"/>
<parameter id="TS1_AdoMet_Ka3_exp" value="1.09" units="dimensionless"/>
<parameter id="TS1_AdoMet_Ka4_exp" value="140" units="mole2_per_litre2"/>
</listOfParameters>
</kineticLaw>
</reaction>
<reaction id="Vcgs" reversible="false">
<listOfReactants>
<speciesReference species="PHser"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="Cysta"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="CGS"/>
<modifierSpeciesReference species="Cys"/>
<modifierSpeciesReference species="Phosphate"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
  <times/> <ci>c1</ci> <ci> CGS </ci> <ci> PHser </ci>
  <apply>
    <divide/>
    <apply>
      <divide/>
      <ci>CGS_kcat_exp </ci>
      <apply>
        <plus/>
        <cn>1</cn>
        <apply>
          <divide/>
          <ci>CGS_Cys_Km_exp </ci>
          <ci>Cys</ci>
        </apply>
      </apply>
    </apply>

    <apply>
      <plus/>
      <apply>
        <times/>
        <apply>
          <divide/>
          <ci>CGS_Phser_Km_exp </ci>
          <apply>
            <plus/>
            <cn>1</cn>
            <apply>
              <divide/>
              <ci>CGS_Cys_Km_exp</ci>
              <ci>Cys</ci>
            </apply>
```

```
          </apply>
        </apply>
        <apply>
          <plus/>
          <cn>1</cn>
          <apply>
            <divide/>
            <ci>Phosphate</ci>
            <ci>CGS_Phosphate_Ki_exp</ci>
          </apply>
        </apply>
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S5 DHDPS1, DHDPS2 and HSK cloning, overexpression and purification procedures

The position of the transit peptide cleavage site of DHDPS was defined by analogy with the *N*-terminus of wheat DHDPS validated by *N*-terminal sequencing (Kaneko *et al.*, 1990). The codon corresponding to Ala38 (DHDPS1) and Ala39 (DHDPS2) in the predicted sequences of the full length proteins were replaced by an initiating Met. The probable position of the transit peptide cleavage site for HSK was identified using the ChloroP program. The codon corresponding to Ala37 in the predicted sequences of the full length protein was replaced by an initiating Met. The cDNA sequences corresponding to the predicted mature proteins were amplified by PCR using an *Arabidopsis thaliana* cDNA library (Elledge *et al.*, 1991) The oligonucleotides contained restriction sites (BspHI and EcoRI for DHDPS1, NcoI and XhoI for DHDPS2 and HSK) for cloning of the PCR fragments into pet23 d(+) vector. BL21 pLys(S) bacteria were used for expression of the recombinant proteins. Transformed bacteria were plated on LB agar supplemented with carbenicillin (100 μg/ml) and chloramphenicol (34 μg/ml) and grown overnight at 37°C. Colonies were transferred into 15 ml of LB media supplemented with the antibiotics and growth was carried out at 37°C. Saturated cultures were transferred into 800 ml LB media supplemented with the antibiotics and growth was continued for 3 hours at 37°C. IPTG was added (0.4 mM) when A_{600nm} of the culture reached 0.6. Growth was stopped after 4 hours for HSK and continued for 18 hours at 20°C for DHDPS. Bacteria were collected by centrifugation (15 minutes, 2000 g, 4°C), re-suspended in a lysis buffer (buffer B: 50 mM Hepes pH 7.5, 1 mM EDTA, 1 mM DTT, 10% glycerol (v/v), 5 mM aminocaproic acid and 1 mM benzamidine, 10 ml per litre culture). Lysis buffer was supplemented with 1 mM lysine for DHDPS. Bacteria were sonicated for 20 minutes at 4°C on a Vibracell disruptor. Streptomycin sulfate (0.1% (p/v)) was added to the sonicate in order to precipitate DNA and the solution was centrifuged for 35 minutes at 30000 ***g*** at 4°C. Purifications steps were carried out at room temperature. The first step consisted in an anion exchange chromatography (DEAE EMD 650(M) column, 26 $\times$ 260 mm^2) equilibrated in buffer C (50 mM Hepes, pH 7.5, 1 mM DTT, 1 mM EDTA, 10% glycerol (v/v)). Buffer C was supplemented with 1 mM Lysine for DHDPS. Proteins were eluted by a gradient of KCl

in buffer C. The anion exchange purification step was sufficient to obtain highly pure HSK enzyme. DHDPS fractions were loaded on HiPrep 16/60 Sephacryl S-200 column (Pharmacia) equilibrated with buffer D (20 mM Hepes, pH 7.5, 100 mM KCl, 1 mM lysine and 10 % glycerol (v/v)). Pure proteins in the elution buffer were concentrated using Centricon devices up to 50–200 μM (on a monomer basis), and aliquots were quickly frozen in N_2 and stored at –80°C for several months without any loss of activity.

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