Supplementary Information to: Low affinity uniporter carrier proteins can increase net substrate uptake rate by reducing efflux

Evert Bosdriesz\textsuperscript{1, 3, +}, Meike T Wortel\textsuperscript{1, 4, +}, Jurgen R Haanstra\textsuperscript{1}, Marijke J Wagner\textsuperscript{1}, Pilar de la Torre Cortés\textsuperscript{2}, and Bas Teusink\textsuperscript{1,*}

\textsuperscript{1}Systems Bioinformatics, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), VU University, Amsterdam, De Boelelaan 1108, 1081HZ, The Netherlands
\textsuperscript{2}Department of Biotechnology, Delft University of Technology, Delft, The Netherlands
\textsuperscript{3}Present address: Division of Molecular Carcinogenesis, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
\textsuperscript{4}Present address: Centre for Ecological and Evolutionary Synthesis (CEES), the Department of Biosciences, University of Oslo, Blindernveien 31, 0371 Oslo, Norway
\textsuperscript{*}b.teusink@vu.nl
\textsuperscript{+}These authors contributed equally
Supplementary Text

Symmetric transport model

Derivation of the rate equation of symmetric transport in terms of first order rate constants

The transporter can be in any of four states, the binding-site facing outward, with and without substrate bound, \( e_x \) and \( e_e \), respectively, and inward facing with and without substrate bound, \( e_i \) and \( e_I \). Assuming that binding and unbinding is much faster than the movement of the binding site over the membrane, we can use the quasi-steady state approximation for the fraction of carriers that have substrate bound to them, both inside and outside,

\[
es_x = \frac{\frac{x}{K_D}}{1 + \frac{x}{K_D}} e_{x,\text{tot}},
\]

\[
e_x = \frac{1}{1 + \frac{x}{K_D}} e_{x,\text{tot}},
\]

where \( K_D \) is the substrate-transporter dissociation constant and \( e_{x,\text{tot}} = e_x + e_e \) is the total number of transporters with their binding site facing the \( x \) site of the membrane (i.e. \( x = e \) or \( x = i \)).

By definition, in steady state \( e_{x,\text{tot}} \) is constant. This gives rise to the equality

\[
k_2 e_x + k_4 e_e = k_2 e_i + k_4 e_i.
\]

Defining

\[
\sigma_x \equiv \frac{k_2 \frac{x}{K_D} + k_4}{1 + \frac{x}{K_D}}
\]

and solving the steady state condition gives an expression for the total amount of outward and inward facing carriers, normalized to the total amount of transporters:

\[
e_{x,\text{tot}} = \frac{\sigma_i}{\sigma_e + \sigma_i} e_{x,\text{tot}},
\]

\[
e_{i,\text{tot}} = \frac{\sigma_e}{\sigma_e + \sigma_i} e_{i,\text{tot}}
\]

The net uptake rate is then given by:

\[
v = k_2 [e_x - e_i] = k_2 \left[ \frac{s_x}{K_D} - \frac{s_i}{K_D} \right] \left( \frac{1}{\sigma_e + \sigma_i} e_{x,\text{tot}} \right)
\]

Filling in the \( \sigma_s \), the term within the square brackets reduces to

\[
\frac{k_4}{K_D} (s_e - s_i)
\]

and the denominator to

\[
2k_4 + (k_2 + k_4) \frac{s_e}{K_D} + (k_2 + k_4) \frac{s_i}{K_D} + 2k_2 \frac{s_e \cdot s_i}{K_D^2}.
\]

Hence, the rate equation in terms of the first order rate constants is given by

\[
v = e_{x,\text{tot}} \frac{\frac{k_2}{2k_0} (s_e - s_i)}{1 + \frac{k_2 + k_4}{2k_0 K_D} e_x + \frac{k_2 + k_4}{2k_0 K_D} e_i + \frac{k_2 s_e \cdot s_i}{k_4 K_D^2}}
\]

Defining the macroscopic kinetic parameters

\[
k_{cat} \equiv \frac{k_2 k_4}{k_2 + k_4}
\]

\[
K_M \equiv \frac{2k_4 K_D}{k_2 + k_4}
\]

\[
\alpha = \frac{4k_2 k_4}{(k_2 + k_4)^2}
\]
than gives the rate equation

\[ v = e_{\text{tot}} \frac{k_{\text{cat}}}{K_M} \left( \frac{s_e - s_i}{s_e + s_i + \frac{\alpha s_e \cdot s_i}{K_M^2}} \right) \]  

(S8)

**Derivation of the optimal affinity, \( K_M^{\text{opt}} \)**

In order to find the optimal \( K_M, K_M^{\text{opt}} \), we simply take the derivative of \( v \) with respect to \( K_M \) and set that to zero. Since we have

\[ \frac{dv}{dK_M} = -e_{\text{tot}}k_{\text{cat}} \frac{1}{K_M^2} (s_e - s_i) \left( 1 + \frac{2s_e \cdot s_i}{K_M^2} - 2\alpha \frac{s_e \cdot s_i}{K_M} \right) \left( 1 + \frac{s_e}{K_M} + \frac{s_i}{K_M} + \frac{\alpha s_e \cdot s_i}{K_M^2} \right)^2, \]  

(S9)

\( K_M^{\text{opt}} \) is found by solving

\[ 0 = 1 + \frac{s_e \cdot s_i}{K_M^2} - 2\alpha \frac{s_e \cdot s_i}{K_M}. \]  

(S10)

The physical (i.e. positive) solution to this quadric equation is

\[ K_M^{\text{opt}} = \sqrt{\alpha \cdot s_e \cdot s_i} \]  

(S11)

In comparison, the reversible Michaelis-Menten rate equation*,

\[ v_{\text{MM}} = e_{\text{t}} \frac{k_{\text{cat}}}{1 + \frac{s_e}{K_M} + \frac{s_i}{K_M}} \]  

(S12)

does not have an optimal affinity. Reducing the \( K_M \) will always increase the rate.

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*Here, we use \( K_{eq} = 1 \) and assume symmetry between \( s_e \) and \( s_i \) release.
The non-symmetric carrier model

In this section we study if the conclusions in the main text hold when the assumptions underlying the simplification of the rate equation are dropped. The more general rate equation describing the net transport rate of a facilitated diffusion process takes the following form (equation IX-46 in \(i^2\)):

\[
v = e_j \frac{k_{cat} \left( s_e - s_i \right)}{1 + \frac{s_e}{K_{M,e}} + \frac{s_i}{K_{M,i}} + \alpha \frac{s_e}{K_{M,e}} \frac{s_i}{K_{M,i}}},
\]

(S13)

where the macroscopic kinetic parameters, \(k_{cat}, K_{M,e}, K_{M,i}\) and \(\alpha\), can be expressed in terms of the first order rate constants.

\[
k_{cat} = \frac{k_{1,f}k_{2,f}k_{3,f}k_{4,f}}{k_{1,f} \left( k_{2,f}k_{3,f} + k_{2,f}k_{4,f} + k_{3,f}k_{4,f} \right)}
\]

(S14a)

\[
K_{M,e} = \frac{\left( k_{4,f} + k_{4,e} \right) \left( k_{1,e}k_{2,e} + k_{1,e}k_{3,e} + k_{2,e}k_{3,e} \right)}{k_{3,f} \left( k_{1,e}k_{2,e} + k_{1,e}k_{4,e} + k_{2,e}k_{4,e} + k_{3,f} \right)}
\]

(S14b)

\[
K_{M,i} = \frac{\left( k_{2,f} + k_{2,i} \right) \left( k_{4,f} + k_{4,i} \right) \left( k_{1,i}k_{2,i} + k_{1,i}k_{3,i} + k_{2,i}k_{3,i} \right)}{\left( k_{2,f}k_{3,f} + k_{2,f}k_{4,f} + k_{3,f}k_{4,f} \right) \left( k_{1,i}k_{2,i} + k_{1,i}k_{4,i} + k_{2,i}k_{4,i} + k_{3,f} \right)}
\]

(S14c)

\[
\alpha = \frac{k_{2,f}k_{3,f} + k_{2,f}k_{4,f} + k_{3,f}k_{4,f}}{k_{1,i}k_{2,i} + k_{1,i}k_{4,i} + k_{2,i}k_{4,i}} + \alpha \frac{s_e}{K_{M,e}} \frac{s_i}{K_{M,i}}
\]

(S14d)

Furthermore, the first order rate equations are related through the equilibrium constant. Since we are considering facilitated diffusion, no free energy dissipation is coupled to the transport process, i.e. \(K_{eq} = 1\), and we have:

\[
1 = K_{eq} \equiv \frac{k_{1,f}k_{2,f}k_{3,f}k_{4,f}}{k_{1,e}k_{2,e}k_{3,e}k_{4,e}}.
\]

(S15)

This poses a constraint on the first order rate constants. Practically, this means that a mutation that affects e.g. the strength of extracellular substrate to carrier binding must also affect some of the other steps in the transport cycle (e.g. intracellular substrate release). Combined with the complicated dependency of the macroscopic parameters on the first order rate constants, an analytical approach is unfeasible. We therefore employed a parameter sampling approach to gauge to what extent our conclusions about the rate-affinity trade-off and the substrate efflux hypothesis are valid for this more general rate equation.

The rate-affinity trade-off

As in the main text, the parameter sampling approach gives a mixed picture about the theoretical underpinnings of the rate-affinity trade-off. Figure S1 shows scatterplots of \(k_{cat}\) versus affinity (defined as \(1/K_{M,e}\)) for randomly sampled sets of first order rate constants, with a number of different constraints assumed for some of these. In the absence of any constraints, there is a clear negative correlation between the \(k_{cat}\) and the affinity (figure S1A). However, this is not a true Pareto-front, as it appears as though there is always a possibility that the \(k_{cat}\) is enhanced without reducing the affinity (or vice versa). The fact that not the whole \(k_{cat} - 1/K_{M}\) space is filled is due to the finite numbers of samples rather than due to a true constraint. On the other hand, if we assume that there is a (biophysical) limitation on the rate of substrate-transporter binding (\(k_{1,f}\)), e.g. the diffusion limit, we do find a true Pareto front (figure S1B), the location of which depends on the actual maximal \(k_{1,f}\)-value. However, this conclusion does not hold if other rate constants are assumed to have some biophysical limit, as shown by the examples of restricted \(k_{2,f}\) (figure S1C) or a restricted substrate-transporter dissociation constant \(K_{D,e} \equiv k_{1,e}/k_{1,f}\), figure S1D). All in all, there are reasonable theoretical arguments to be made for a rate-affinity trade-off, but the logic is not water-tight.

Enhanced uptake by reduced efflux

In the analysis in the main text above we made the biologically motivated, simplifying assumption that the transporter is symmetric. However, our reasoning does not critically depend on this symmetry, since it is a general property of this scheme that the substrate and product bind to different states of the transporter. Moreover, since all first order rate are interdependent through constraint (S15), \(K_{M,i}\) and \(K_{M,e}\) are expected to be correlated. To test this, we randomly sampled all first order rate constants from a log-normal distribution and rescaled them such that \(K_{eq} = 1\) and \(k_{cat} = 1\) (for details, cf. Appendix ). These parameters were used to calculate the \(K_{M,e}, K_{M,i}\) and the net steady state uptake rate \(J\) under conditions of high an low external substrate (\(s_e = 100\) and \(s_e = 1\), respectively). The results are depicted in figure S2. Indeed, \(K_{M,e}\) and \(K_{M,i}\) are correlated, albeit not strongly (Spearman correlation = 0.64, Figure S2A). More importantly, however, there appears to be an \(s_e\)-dependent optimal affinity (figure S2B). Furthermore, the set of parameters that has the highest \(J\) under low substrate conditions, performs relatively poorly under high substrate conditions (large, light red dot), and vice versa (blue dot).
Parameter sampling procedure

Sets of parameters were constructed by drawing the first order rate constant randomly from a log-normal distributions. This was done in a way such that constraint (S15) is satisfied. These parameter sets were used to calculate the steady state uptake rate (given by equation (S13)) and macroscopic kinetic parameters (given by equation (S14)). The parameter sampling was performed in Wolfram Mathematica 9.0 using the functions RandomVariate and LogNormalDistribution, which has the probability density function (PDF):

$$\exp\left(\frac{(\ln(x) - \mu)^2}{2\sigma^2}\right)$$

$$\sqrt{2\pi \sigma \mu}$$

(S16)

Rate affinity trade-off

To generate the data depicted in figure S1, for each subfigure 10 000 parameter sets were constructed. Each parameter set was constructed in the following way:

- Two sets of four numbers, $\mathcal{X} \equiv \{x_1, x_2, x_3, x_4\}$ and $\mathcal{Y} \equiv \{y_1, y_2, y_3, y_4\}$ were randomly drawn from a log-normal distribution given by the PDF (S16) with $\mu = 0$ and $\sigma = 2$.

- For figure S1A, there are no restrictions on individual rate constants. The set of forward rate constants, $\mathcal{K}_f \equiv \{k_{1f}, k_{2f}, k_{3f}, k_{4f}\}$ is just given by the first set, $\mathcal{K}_f = \mathcal{X}$. To get the reverse rate constants $\mathcal{K}_r \equiv \{k_{1r}, k_{2r}, k_{3r}, k_{4r}\}$, $\mathcal{Y}$ needs by be rescaled by a factor

$$a = \prod_{i=1}^{4} \left(\frac{x_i}{y_i}\right)^{1/4}.$$ 

(S17)

This is to ensure that $K_{eq} = 1$. Hence, $\mathcal{K}_r = a \cdot \mathcal{Y}$.

- For figure S1B we also need to ensure that $k_{1f}$ is restricted to some constant value $c$. We set the first order forward rate constants to $\mathcal{K}_f = \frac{e^{x_1}}{x_1} \cdot \mathcal{X}$ and $\mathcal{K}_r = \frac{e^{x_2}}{x_2} \cdot \mathcal{Y}$. Similarly, for figure S1C we need to restrict $k_{2f}$ to $c$. We use $\mathcal{K}_f = \frac{e^{x_3}}{x_3} \cdot \mathcal{X}$ and $\mathcal{K}_r = \frac{e^{x_4}}{x_4} \cdot \mathcal{Y}$.

- For figure S1D, the $K_{D,e}$ is fixed to a constant value $c$. Here, we used for the forward rate constant simply $\mathcal{K}_f = \mathcal{X}$. Since $K_{D,e} \equiv k_{1r}/k_{1f}$, we set $k_{1r} = c \cdot x_1$. Defining $\beta = \left(\frac{x_2 x_3 x_4}{y_2 y_3 y_4 c}\right)^{1/3}$ and setting $\{k_{2r}, k_{3r}, k_{4r}\} = \beta \cdot \{y_2, y_3, y_4\}$ additionally ensures $K_{eq} = 1$.

Enhanced uptake by non-symmetric low affinity transporters

Figures S2 A and B are constructed from the same 10000 parameter sets. Each set was constructed such that $k_{\text{cat}} = 1$ and $K_{eq} = 1$ as follows:

- Two sets of four numbers, $\mathcal{X} \equiv \{x_1, x_2, x_3, x_4\}$ and $\mathcal{Y} \equiv \{y_1, y_2, y_3, y_4\}$ were randomly drawn from a log-normal distribution given by the PDF (S16) with $\mu = 0$ and $\sigma = 2$.

- The set $\mathcal{Y}'$ is obtained by rescaling $\mathcal{Y}$ by a factor

$$a = \prod_{i=1}^{4} \left(\frac{x_i}{y_i}\right)^{1/4}$$

(S18)

to obtain $\mathcal{Y}' \equiv a \cdot \mathcal{Y}$. This ensures $\prod_{i=1}^{4} x_i/y_i' = 1$.

- Both sets are rescaled by a factor $1/k_{\text{cat}}$, as defined in equation (S7a) with $k_{if} \rightarrow x_i$ and $k_{ir} \rightarrow y_i d_e$.

$$k_{\text{cat}} = \frac{x_1 x_2 x_3 x_4}{x_1 (x_2 x_3 + x_2 x_4 + x_3 x_4 + x_3 x_4)}$$

(S19)

This rescaling is done to ensure that $k_{\text{cat}} = 1$ for each parameter set. The first order rate constants are thus $\mathcal{K}_f = \frac{1}{k_{\text{cat}}} \mathcal{X}$ and $\mathcal{K}_r = a k_{\text{cat}} \mathcal{Y}$.
### Supplementary Tables

| Name         | Genotype                                                                                                                                  | Relevant phenotype                                                                                   | Origin                      |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-----------------------------|
| CEN.PK113-7D | MATa URA3 HIS3 LEU2 TRP1 MAL2-8c SUC2                                                                                                        | Prototrophic control strain                                                                        | Euroscarf                   |
| EBY.VW4000   | MATa ura3 his3 leu2 trp1 MAL2-8c SUC2 hxt13Δ::loxP hxt15Δ::loxP hxt16Δ::loxP hxt14Δ::loxP hxt12Δ::loxP hxt9Δ::loxP hxt11Δ::loxP hxt10Δ::loxP hxt8Δ::loxP hxt514Δ::loxP hxt2Δ::loxP hxt367Δ::loxP gal2Δ stl1v::loxP agt1Δ::loxP ydl247wΔ::loxP yjr160cΔ::loxP | HXT null strain, auxotrophic for uracil, leucine, histidine and tryptophan. Does not grow on glucose as sole carbon source | Wieczorke et al. (1999)    |
| IMX746       | MATa ura3 MAL2-8c SUC2 hxt13Δ::loxP hxt15Δ::loxP hxt16Δ::loxP hxt14Δ::loxP hxt12Δ::loxP hxt9Δ::loxP hxt11Δ::loxP hxt10Δ::loxP hxt8Δ::loxP hxt514Δ::loxP hxt2Δ::loxP hxt367Δ::loxP gal2Δ stl1v::loxP agt1Δ::loxP ydl247wΔ::loxP yjr160cΔ::loxP can1::LEU2-HIS3-TRP1 | HXT null strain with a single auxotrophy for uracil                                              | This study                   |
| IMI335       | MATa MAL2-8c SUC2 hxt13Δ::loxP hxt15Δ::loxP hxt16Δ::loxP hxt14Δ::loxP hxt9Δ::loxP hxt11Δ::loxP hxt10Δ::loxP hxt8Δ::loxP hxt514Δ::loxP hxt2Δ::loxP hxt367Δ::loxP gal2Δ stl1v::loxP agt1Δ::loxP ydl247wΔ::loxP yjr160cΔ::loxP can1::LEU2-HIS3-TRP1 ura3::HXT7_T213N-URA3. | Prototrophic HXT null strain expressing HXT7_T213N                                              | This study                   |
| IMI337       | MATa MAL2-8c SUC2 hxt13Δ::loxP hxt15Δ::loxP hxt16Δ::loxP hxt14Δ::loxP hxt9Δ::loxP hxt11Δ::loxP hxt10Δ::loxP hxt8Δ::loxP hxt514Δ::loxP hxt2Δ::loxP hxt367Δ::loxP gal2Δ stl1v::loxP agt1Δ::loxP ydl247wΔ::loxP yjr160cΔ::loxP can1::LEU2-HIS3-TRP1 ura3::HXT7_T213T-URA3. | Prototrophic HXT null strain expressing HXT7_T213T                                              | This study                   |

**Table S1.** *Saccharomyces cerevisiae* strains used in this study
### Overlapping sequences for homologous recombination

| Name            | Sequence 5' --> 3'                                                                 | Purification |
|-----------------|----------------------------------------------------------------------------------|--------------|
| 5890_TRP1 fw    | AATAAAGCTCATATCTATGCTACAACATCCAAAATTTGTCCC AAAAGCTTTCTGGTTCAAGACCGAGTTAGGGACAG | HPLC         |
| 5891_TRP1 rv    | GTGCCCTATGAGTCTGGCCGGAATGTCTGCCCCTGCATACGC CATGCCCTCACATATGGCTCTACCTGGTTATG | HPLC         |
| 2335_HIS3 fw    | ACTATATGACGAGCCAGCTCTGAGCACGAGCTTACGCACTCCTGCGCTCTCTGCTAGACACTG | HPLC         |
| 2336_HIS3 rv    | GTGACACATGGTCTGGCTGAATCTTTTGAGACGCCGAATATCCTAATACCCCTGGCAGCGATACCTGC | HPLC         |
| 5892_LEU2 fw    | CAAGCTTGGAGAAGAAGCCGACATAGAGGAAAGCAT ATGTAATGGACCGGGTGTCTACAGTGCAGGGCCATCG | HPLC         |
| 7052_LEU2 rv    | ATGACAAATTCAAAGAGACGCGCGACATAGAGGAAAGCAT ATGTAATGGACCGGGTGTCTACAGTGCAGGGCCATCG | HPLC         |

### Overlapping sequences for Gibson assembly

| Name            | Sequence 5' --> 3'                                                                 | Purification |
|-----------------|----------------------------------------------------------------------------------|--------------|
| 6290_prRS406 fw | GCTCTTATTGACACACCTACCCCTACCCCGACATCCACAGAAACACAGGGGAAACG | HPLC         |
| 6291_prRS406 rv | GTTGGTGACGCTCAACATTCAACGCTAGATCACTACACCCGCAAGGGAATACAC | HPLC         |
| 6292_TDHS3p fw  | ATATATCAGATATACCTCTTAGCGGTGATGATAGGTGTTGAGACACGCTACACG | HPLC         |
| 6293_TDHS3p rv  | TTGTCTGCAGATAGAGCGGAAATTCTGCTACATCGATTTTAGGGGACGGGAACG | HPLC         |
| 6294_HXT7 fw    | GTGTCGAAATAACACATATAAAACACAAATGTCAGATTACACACCTGATGC | HPLC         |
| 6295_HXT7 rv    | TCTTCTGCAGTATCCCTACGTCTGTGCAGTCATACGAGGAGGTGATTGGAATACG | HPLC         |

### Diagnostic primers for homologous recombination

| Name            | Sequence 5' --> 3'                                                                 | Purification |
|-----------------|----------------------------------------------------------------------------------|--------------|
| 5894_diag CAN1 fw | TCGGGAGCAAGATGGTGTG GAAGGCATGCATCATGATCATGATGTGATGTGATGGTGTGATGTGATGGTGTGATG | Desalted    |
| 5895_diag TRP1 rv | GCCGTAATCTTGAACGAC | Desalted    |
| 5896_diag TRP1 rv | CCGGAGGTTGGAGAACAG | Desalted    |
| 5897_diag HIS3 rv | GGCGTCTTGGCTCTTATC | Desalted    |
| 5898_diag HIS3 rv | TCCCTCACCACGAGGTTTC | Desalted    |
| 5899_diag LEU2 rv | CTGTCCGCAGGAATGTAAG | Desalted    |
| 5900_diag LEU2 rv | TCGGCTGTAATTCCTTGACC | Desalted    |
| 5901_diag CAN1 rv | AGAAGAGTGATGGGAGGAC | Desalted    |

### Diagnostic primers for Gibson assembly

| Name            | Sequence 5' --> 3'                                                                 | Purification |
|-----------------|----------------------------------------------------------------------------------|--------------|
| 6034_diag TDHS3p fw | CTTCTGCTCTCTGATTTGG | Desalted    |
| 1852_diag HXT7 rv | GTACAATGCTGGCTCATGCTAGG | Desalted    |
| 1693_diag URA3 rv | GCTGTCTAATCTGCTATGATC | Desalted    |
| 2613_diag TDHS3p rv | CGATGCTACGAGGGAATATCTAGTATATAG | Desalted    |
| 3795_diag ADH1t fw | ATGACGCTGAGGAGGAGAATTCATTATGATTTAATG | Desalted    |
| 3228_diag prRS406 rv | GCGGATATAAAGTCTGAGGCAG | Desalted    |

Table S2. Primers used in this study
| Name                        | Relevant characteristics                                                                 | Origin                      |
|-----------------------------|------------------------------------------------------------------------------------------|-----------------------------|
| Hxt7mnx-pVT_T213N           | plasmid pVT-102-U expressing the HXT7_T213N isoform under the ADH1 promoter               | Kasahara et al (2011)       |
| Hxt7mnx_pVT_T213T           | plasmid pVT-102-U expressing the HXT7_T213T isoform under the ADH1 promoter               | Kasahara et al (2011)       |
| pRS405                      | Template for LEU2 gene amplification                                                      | ATCC, Sikorski et al. (1989)|
| pRS406                      | Template for Gibson assembly                                                              | ATCC, Sikorski et al. (1989)|
| pUDI091                     | URA3, pMB1, Sc pTDH3-HXT7_T213N-tADH1                                                     | This study                  |
| pUDI093                     | URA3, pMB1, Sc pTDH3-HXT7_T213T-tADH1                                                     | This study                  |

**Table S3.** Plasmids used in this study
Figure S1. Parameter sampling gives inconclusive picture of potential rate-affinity tradeoff. See Supplementary Information for explanations and details on the parameter sampling procedure.
**Figure S2.** Parameter sampling confirms enhanced uptake by non-symmetric low affinity carriers. The large red (blue) dot in panel B indicate the set parameters that are optimal at low $s$ (high $s$). See Supplementary Information for details on the parameter sampling procedure.

**Figure S3.** Effect of substrate efflux is $V_{\text{max}}$-dependent. Steady-state glycolytic flux relative to the transporters, $J_{\text{glycolysis}}/V_{\text{max,GLT}}$ as a function of $K_{M,\text{GLT}}$ for different values of $V_{\text{max,GLT}}$. A lower $V_{\text{max}}$ reduces the intracellular glucose concentration. As a consequence, the effect of substrate efflux is less pronounced (the steady-state flux is closer to the $V_{\text{max}}$) and the optimal transporter affinity is higher. Due to this effect, the reduced-efflux hypothesis can only be tested using transporters that are comparable both in $k_{\text{cat}}$ and $V_{\text{max}}$. The same model as in Figure 2B was used for these simulations, with [Glucose] = 110mM.
Figure S4. Repair of auxotrophies in the EBY.VW4000 strain. A PCR amplification of the LEU2, HIS3 and TRP1 genes with sequences overlapping either the adjacent cassettes or the integration site. B In vivo assembly of the marker cassettes and integration in the CAN1 locus of EBY.VW4000.