



Figure A1. The regulation function of the *lac* operon with lactose and IPTG, as calculated by numerical integration of equations (A.1)–(A.8).

Table A1. Variables and constants used for modeling the *lac* operon.

Parameter	Definition	Value
$L_{(e)}$	External lactose concentration	
X	External IPTG or TMG	
L	Internal lactose concentration	
A	Allolactose concentration	
R	Active (=DNA binding) LacI concentration	
Z	LacZ concentration	
Y	LacY concentration	
$\beta_{y,z}(L)$	The regulation <i>function</i> expressing the production rate of LacZ (or LacY) as a function of external lactose (or IPTG) concentration L	
R_T	Total amount of repressor (constant)	0.01 μM (10 molecules) [10]
Z_T	Maximal amount of LacZ	50 μM ($\sim 10^4$ molecules) [10]
Y_T	maximal amount of LacY	50 μM ($\sim 10^4$ molecules) [10]
$\tau_{\text{cell-generation}}^{-1}$	Cell generation	30 minutes [17]
$\alpha, \tau_{\text{dilution}}^{-1}$	Dilution rate	$\frac{\ln 2}{\tau_{\text{cell-generation}}} = 2.26 \times 10^{-2} \text{ min}^{-1} \approx \frac{1}{50 \text{ min}}$
$\beta_z (\beta_{\text{WT}}, \beta_{\text{max}})$	Maximal production rate of LacZ	$\sim 10^2 \text{ molecules min}^{-1}$ [16]
$\beta_y (\beta_{\text{WT}}, \beta_{\text{max}})$	Maximal production rate of LacY	$\sim 10^2 \text{ molecules min}^{-1}$ [16]
$K_{\text{TMG-y}}$	Affinity of TMG and LacY	700 μM [53]
$K_{L(e)-y}$	Affinity of external lactose and LacY	400 μM [5]
K_{L-y}	Affinity of (internal) lactose and LacY	1.8 mM [5]
K_{L-Z}	Affinity of (internal) lactose and LacZ	1.4 mM [5]
K_{A-Z}	Affinity of Allolactose and LacZ	1.9 mM [17]
K_{A-R}	Affinity of Allolactose and LacI	6 μM [17]
$K_{\text{IPTG-R}}$	Affinity of IPTG and LacI	Assumed to be 1 μM
$K_{\text{TMG-R}}$	Affinity of TMG and LacI	Assumed to be 6.3 μM
$K_{R-\text{dna}}$	Affinity of LacI and the DNA	10^{-10} – 10^{-11} M [53]
v_y	Velocity of pumping by LacY	3000 molecules min^{-1} [17]
\bar{v}_y	Velocity of efflux by LacY	$v_y/100$ [5]
v_{z1}	Velocity of internal lactose hydrolysis by LacZ	$v_{z1} = 0.9v_y$ [5]
v_{z2}	Velocity of conversion of lactose to allolactose by LacZ	20 000 molecules min^{-1} [17]
v_{z3}	Velocity of allolactose hydrolysis by LacZ	20 000 molecules min^{-1} [17]

relative to the cell cycle due to the fast utilization rates of internal lactose and allolactose by LacZ ($(v_{z1} + v_{z2})[LZ] \gg \tau_{\text{dilution}}^{-1}L$; $v_{z3}[AZ] \gg \tau_{\text{dilution}}^{-1}A$). Hence a steady-state metabolic flow can be assumed above this timescale.

- Equation (A.3) also describes a fast reaction (protein-ligand binding and protein activation), reaching steady state after roughly 1 ms.
- Equations (A.4) and (A.5) describe the production of LacZ and LacY through transcription and translation. They are valid for timescales above 5 min, and will reach half way to steady state only after a cell generation (Binding of

LacI to the DNA will reach steady state after 1s, mRNA lifetime is roughly 2 min, and translation will reach steady state after approximately 5 min. Thus the production rate reaches steady state after 5 min).

Note also that in equation (A.1) we have neglected the efflux component.

In order to be activated, the *lac* operon needs to maintain a small basal level of LacY pumps in order to detect small amounts of lactose. Figure A1 shows the response function for different amounts of lactose, as calculated by numerical integration of equations (A.1)–(A.5).