Designing an irreversible metabolic switch for scalable induction of microbial chemical production

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Motivation

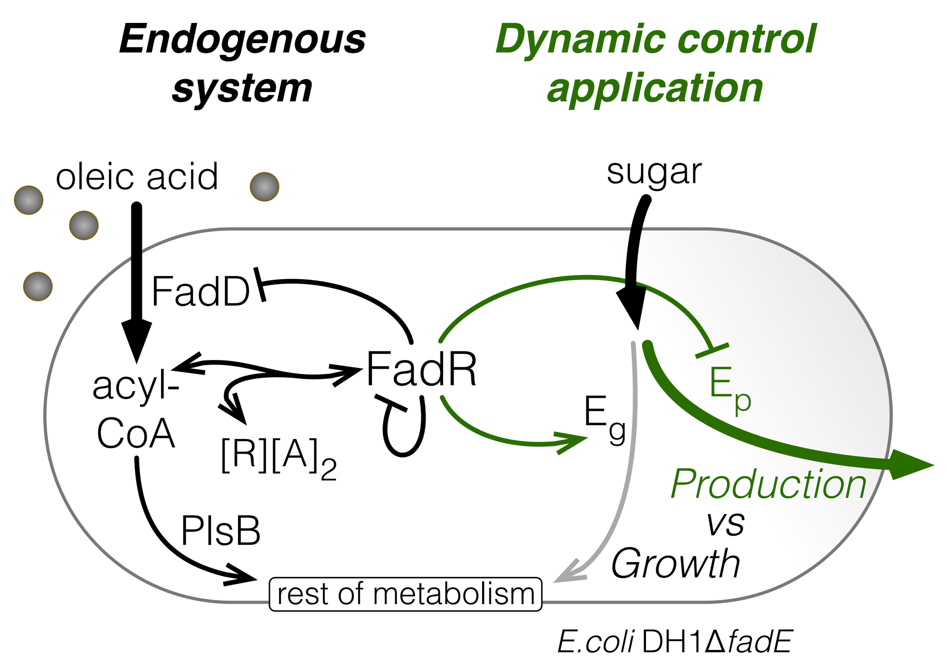
— Inducible genetic circuits can be used to switch cell metabolism from growth to chemical synthesis, overcoming their inherent trade-off

— But, use of costly inducers or need for constant addition, to counter their consumption, limits scalability of inducible chemical production.

Question

— Can we engineer a genetic circuit to switch on and retain production, after temp. induction with a cheap natural nutrient, like oleic acid?

Oleic acid-inducible dynamic control



Mathematical model

Protein expression

$$\frac{dR}{dt} = b_R + P_R(R) - k_f A^2 R + k_r C - \lambda R$$

$$\frac{dD}{dt} = b_D + \frac{a_D}{1+(K_D R)^2} - \lambda D$$

$$\frac{dE_g}{dt} = \frac{a_g K_g R}{1+K_g R} - \lambda E_g$$

Native negative autoreg. (NAR): $P_R(R) = \frac{a_R}{1+K_R R}$

Engineered positive autoreg. (PAR): $P_R(R) = \frac{a_R K_R R}{1+K_R R}$

Reaction kinetics and metabolite dynamics

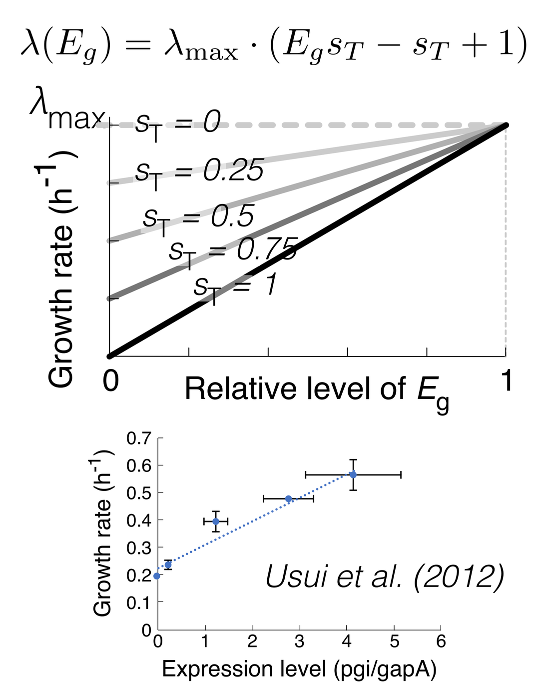
$$r_u = \frac{k_{cat,D}\cdot OA}{K_{m,D}+OA}\cdot D \qquad \frac{dA}{dt} = r_u - r_c - 2\cdot r_{seq} - \lambda A$$

$$r_c = \frac{k_{cat,B}\cdot A}{K_{m,B}+A}\cdot B \qquad \frac{dC}{dt} = r_{seq} - \lambda C$$

$$r_{seq} = k_f A^2 R - k_r C$$

Growth dynamics

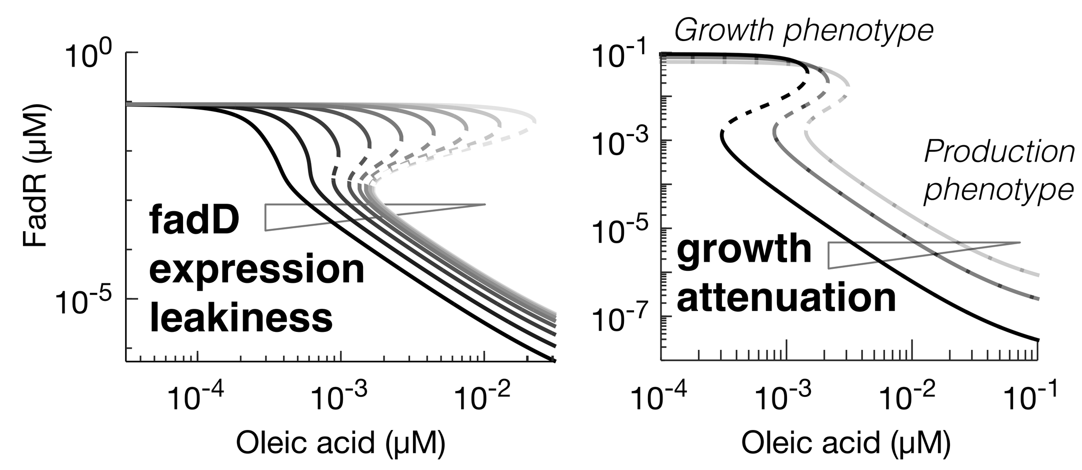
$$\lambda(E_g) = \lambda_{max}\cdot(E_g s_T - s_T + 1)$$



Engineering a bistable metabolic switch

— We can tune the native circuitry to achieve a bistable switch.

— Switch reduces growth, but this reinforces bistable behaviour.



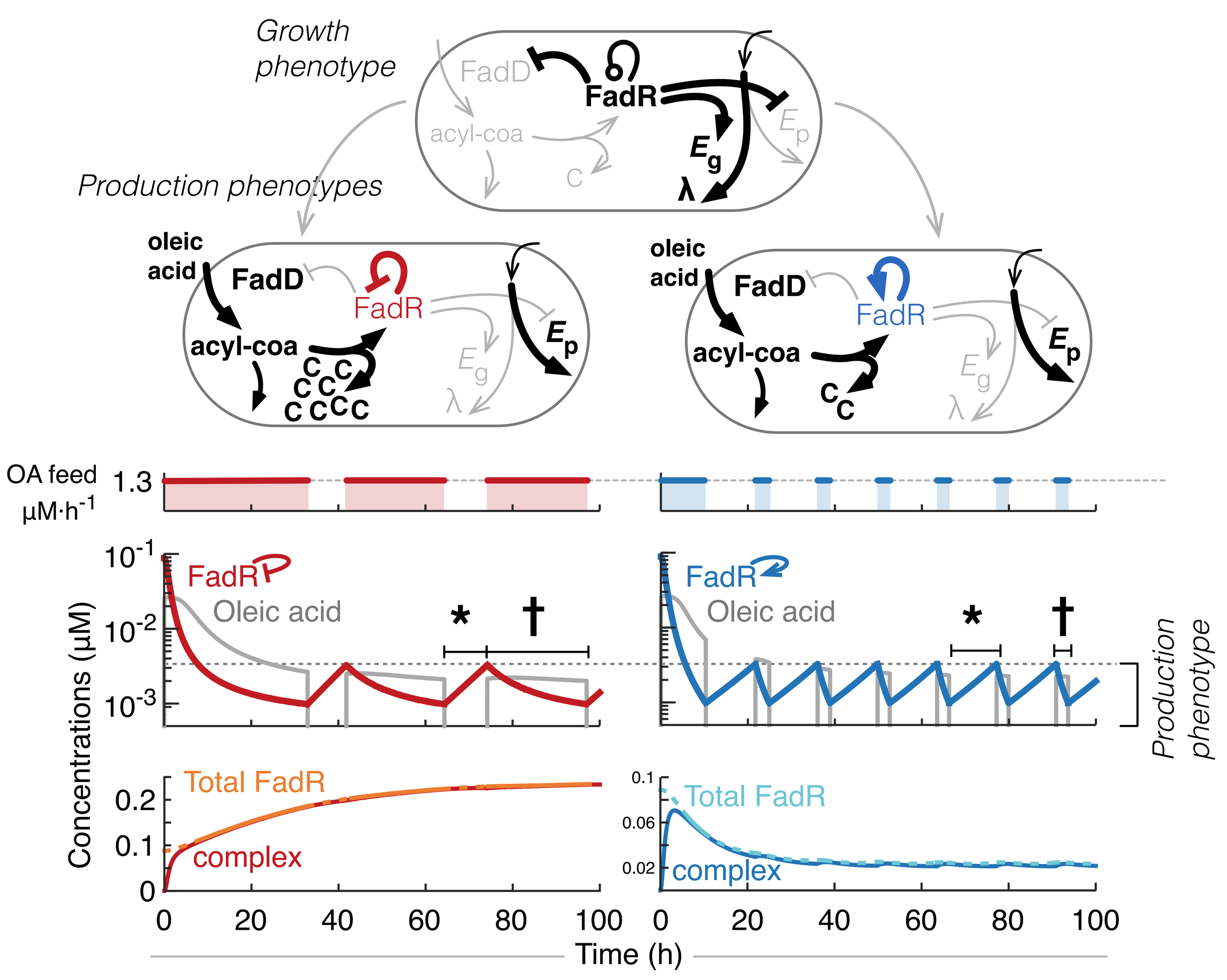
— OA is consumed, so need to constantly add it to retain production.

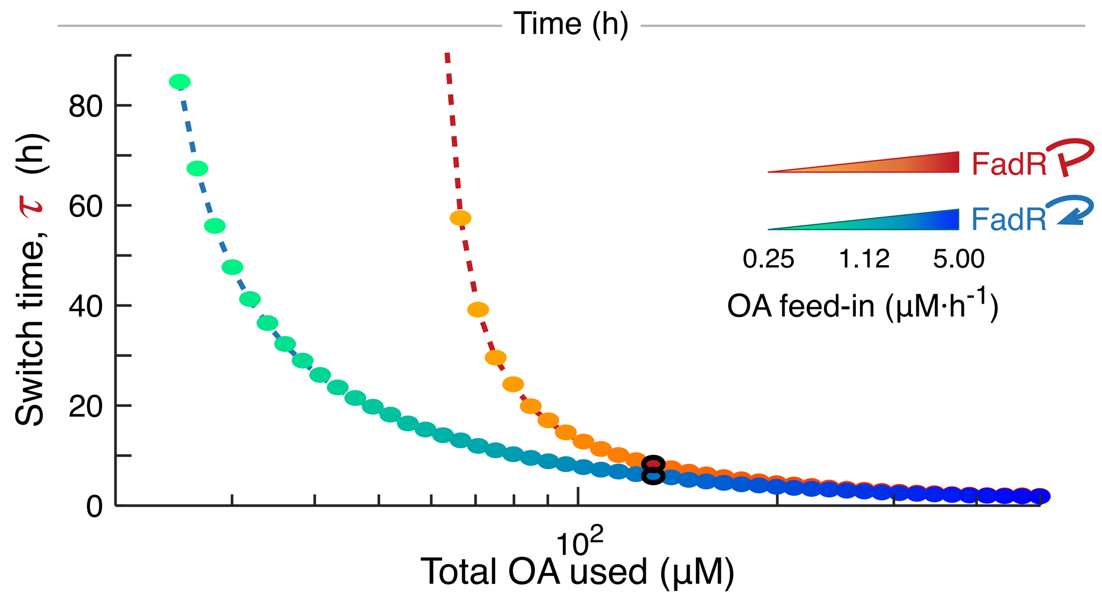
— Changing FadR NAR to PAR significantly cuts total inducer used:

— FadR is not stored and instead diluted away,

* — this slows reversion after OA depletes,

† — and reduces OA additions to retain production.

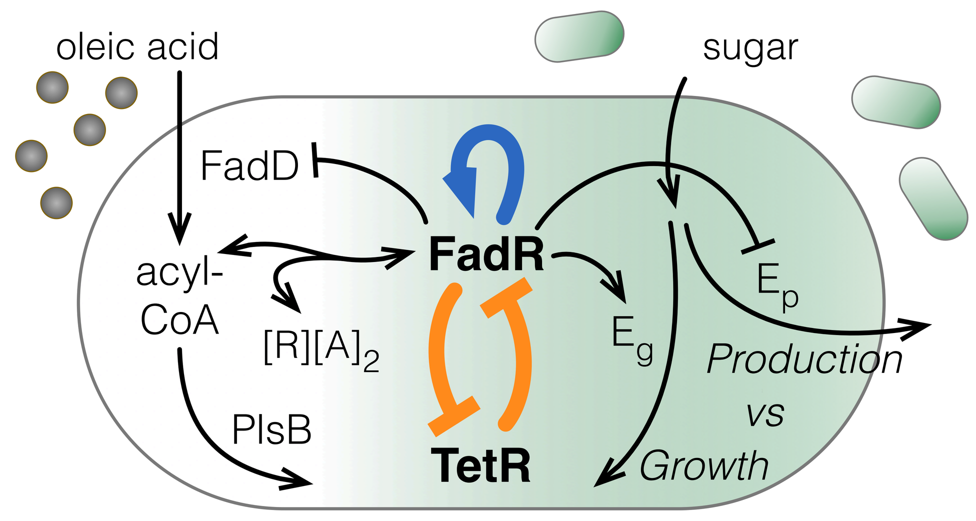




Problem — Intermittent OA addition is costly and limits scalability.

Designing and optimising an irreversible switch

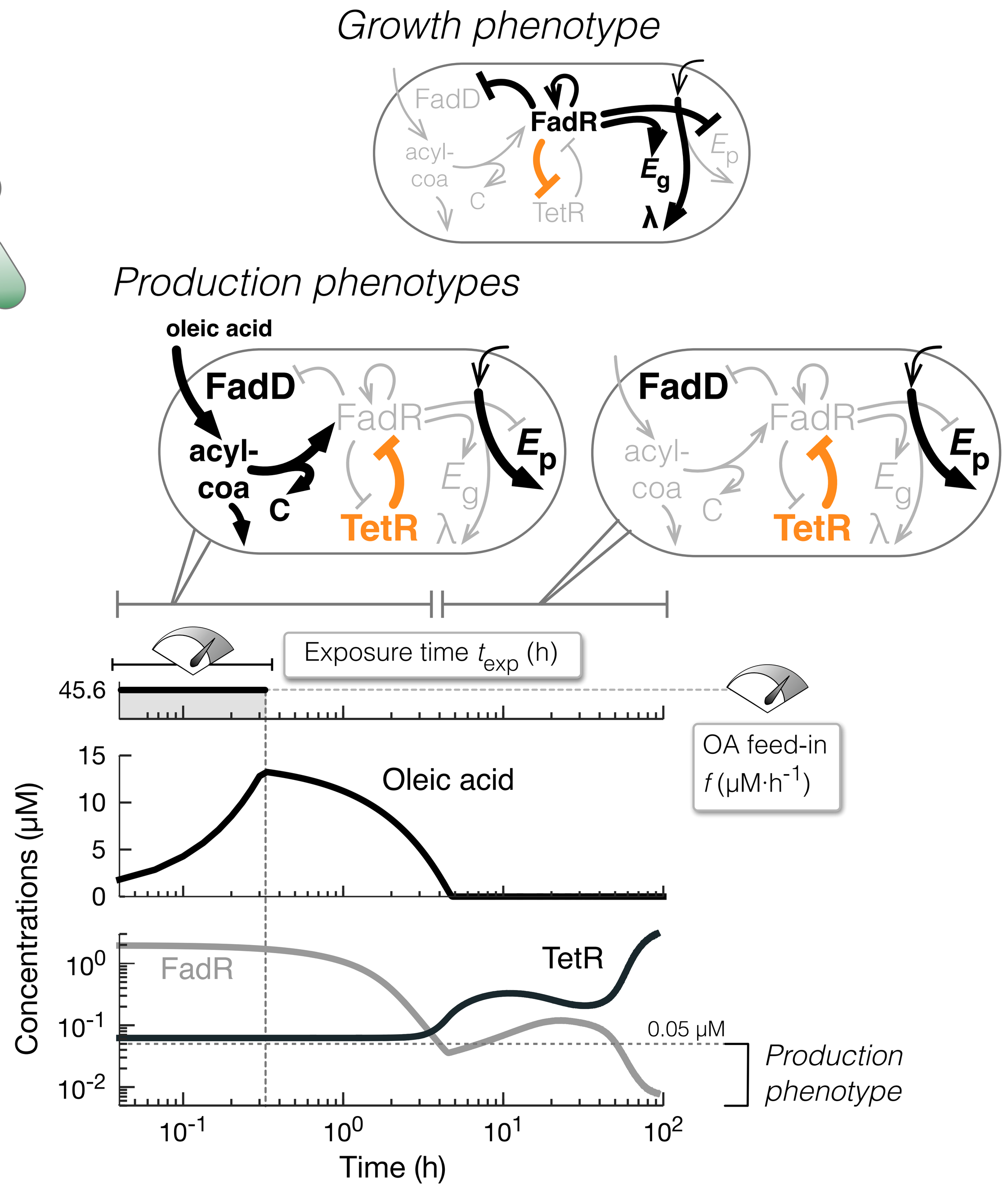
Irreversible OA-inducible switch



— **PAR** dilutes away FadR during induction.

— Augmenting **positive feedback loop** stops further FadR expr.

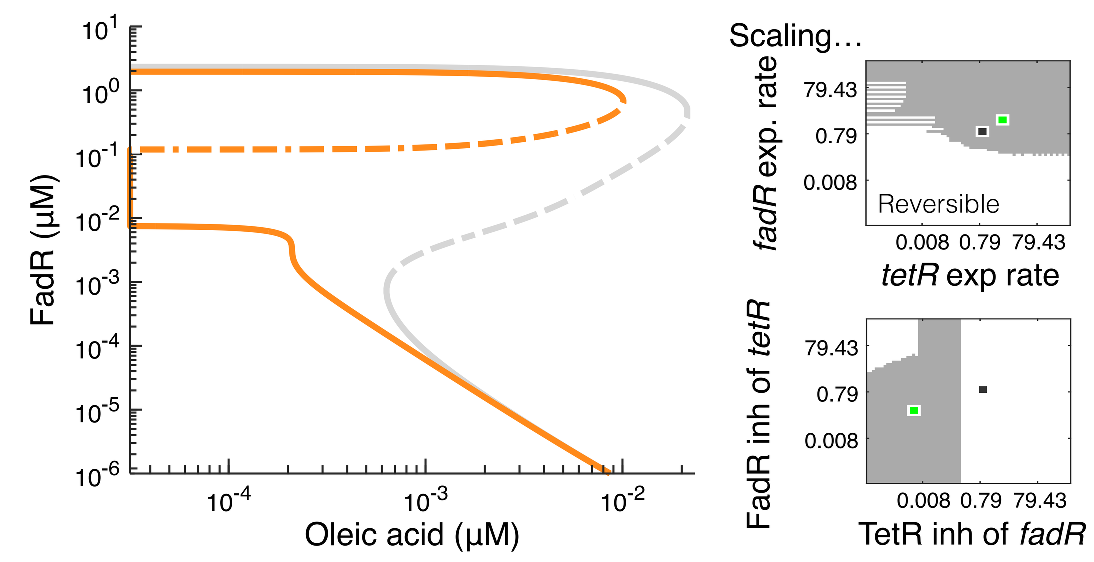
— This **irreversibly locks** cell at **production phenotype**.



— **Key principles** to **engineering irreversible** genetic switch:

(1) strong promoter strengths of FadR and TetR,

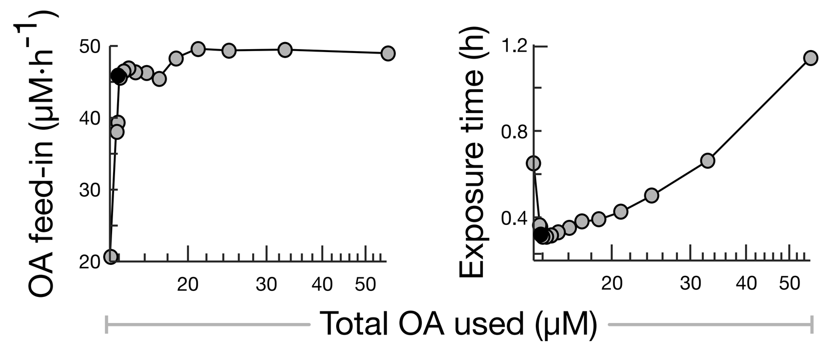
(2) similar but weak inhibitions of each TF on other's expression.

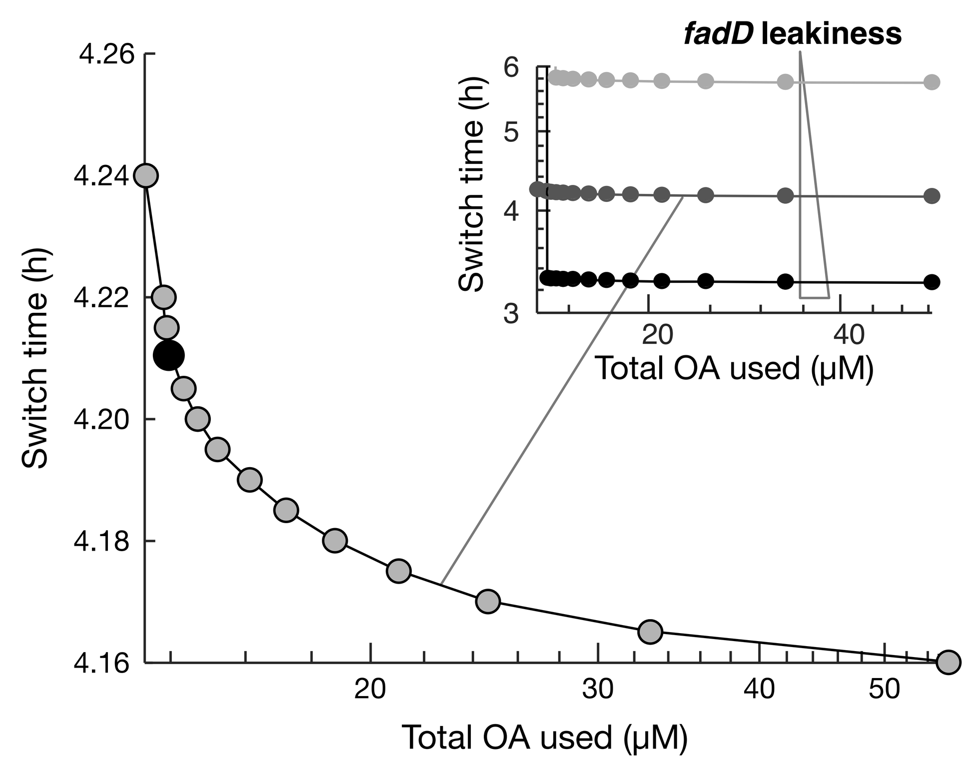


— **Key principles** to **optimising performance**:

(1) min total inducer use
— short induction at high OA conc.

(2) min time to switch to production
— increase leakiness of OA uptake enzyme FadD.

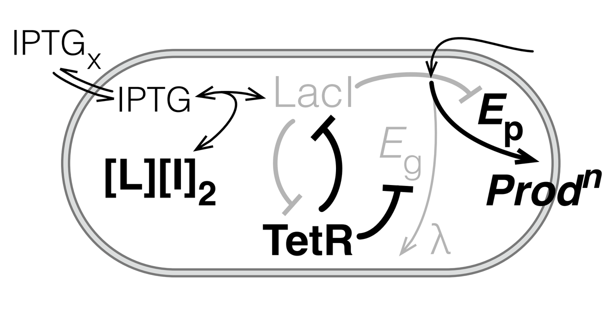




Implications and impacts

— Similar perf. with toggle switch

— But, inducing with nutrients **cheaper**, so **more scalable**



— **Wide applicability**
- many hosts
- many chems

— **General principles** to construct any nutrient ind. irrev. switch

TF	Inducer	Inducer cost *
LacI	IPTG	£ 1055
BetI	Choline	£ 180
FadR	Fatty oleic acid	£ 211
GntR	D-gluconate	£ 22
TreR	Trehalose-6-pi	£ 237
TyrR	L-tyrosine	£ 39

* cost for 25g (≥99%) from Merck, SigmaAldrich.

Take-home messages

— Oleic acid inducible genetic switch can be constructed to irreversibly activate synthesis.

— General design principles — switch can be made for other nutrient-inducible TFs.

— Should be widely applicable — for use in many host cells & synthesis of any product.

— Temporal addition of cheap natural nutrient cuts costs — making induction of microbial chemical production more scalable.

Key references

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